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BLOOD GROUPING TECHNICS IN CLINICAL MEDICINE*

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Introduction

Landsteiner's prediction in 1927 that "human beings have blood group patterns as distinctive and individual as finger prints" certainly has been borne out more and more each year as new blood groups are discovered. With the increasing interest and research being done in the field of blood grouping, there is reason to believe that, in the not too distant future, this may become a reality. (Table 1)

These are the nine blood group systems as we know them today. Since this table was made, two other antigens have been found—little f in the Rh system and Jk^b, the predicted allele of Jk^a.

The clinical importance of the blood group factors is related to their antigenicity in humans. In at least 95 per cent of clinical difficulties the blood groups A, B, and Rh_o (D) are involved, but any of the others in column II may cause clinical problems. By clinical problems we mean blood transfusion reactions and complications in pregnancy resulting in an infant with erythroblastosis fetalis of varying degrees of severity.

Hemolytic transfusion reactions and erythroblastosis fetalis have been reported in the literature resulting from many of the known identified blood group antigens, but their antigenicity varies considerably.

In our discussion this morning we will consider various technics of cross-matching blood. The only essential difference between cross-matching a blood for transfusion and testing the serum of an individual for the presence of abnormal antibodies is that in the latter case, incompatible bloods are matched, as well as compatible ones. The methods and problems are nearly identical in both instances.

*Panel Discussion: ASMT, June 1953, Louisville, Ky.

THE IMPORTANT BLOOD GROUP FACTORS

I	II	III
A-B-O	A B	O
Rh	D	
	C c	
	E e	
M-N-S	M N	
	S s	
Kell	K k	
Duffy	Fy^a Fy^b	
Kidd	Jk^a	
P		P
Lewis		Le^a Le^b
Lutheran		Lu^a

TABLE 1

TESTS FOR THE DIAGNOSIS AND PROGNOSIS OF ERYTHROBLASTOSIS FETALIS

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Erythroblastosis fetalis is a disease of infancy which is the result of a blood group incompatibility between parents—the most common involving the Rh factor in which the mother is essentially Rh-negative (D negative) and the father is essentially Rh positive (D positive). However, we will show later on, that an incompatibility in one or more of four of the other blood group systems may also cause this disease. It is most important to remember that erythroblastosis fetalis is extremely variable in its severity. Therefore, conclusions drawn from a small number of cases are often invalid.

Erythroblastosis fetalis is not a very common disease—it occurs in approximately 1 in 100 pregnancies—but it is responsible for 5 to 10 per cent of all stillbirths, and, if not properly treated, for a similar fraction of all neonatal deaths and all cases of cerebral palsy.

The diagnosis of erythroblastosis fetalis depends upon which blood factor is causing the disease in a particular case. In perhaps 80 per cent of the cases, the Rh_o or D antigen is the causative factor. In the remainder of the cases not due to Rh_o or D, most of the mothers are Rh positive. In these cases, the baby nearly always looks normal at birth, but becomes jaundiced during the first 12 to 24 hours of life. Jaundice is a very important symptom to be watched for closely. Should it occur, the mother's and baby's bloods should be retested for blood group and Rh factor and a direct Coombs' test done on the baby's red cells. If the mother is Rh-positive and the direct Coombs' test is positive, then probably the disease is caused by one of the other blood groups, e.g., Kell, E, c, etc. If the baby's direct Coombs' test is negative and there is an incompatibility in the major blood groups between mother and baby, it is almost certain that it is one of the cases caused by a major blood group incompatibility, e.g., the mother is Group O and the father and baby Group A or B.

INCOMPATIBILTIES THAT MAY RESULT IN ERYTHROBLASTOSIS

Group of Baby	Group of Mother
A	O
B	O
A or AB	B
B or AB	A

Table 2

Natural anti-A and/or anti-B agglutinins occur in all Group O, A and B individuals, except in some newborn infants and are demonstrable in a saline medium. Hyper-immune antibodies are the result of stimulation, either from pregnancy by non-specific antigens, or by injections of specific antigens, such as Witebsky's AB Group Specific Substance. Hyper-immune antibodies are not easily neutralized by the addition of Witebsky's Group Specific Substance to the serum. As might be expected, these are the antibodies which cause erythroblastosis fetalis in an incompatible infant. It is difficult to predict erythroblastosis fetalis on this basis and it is not known, at present, why the Coombs' test is negative. If the mother's serum is shown to contain hyper-immune anti-A or anti-B agglutinins, as the case may be, this is additional evidence that the disease in her newborn infant may have resulted from a major blood group incompatibility. The procedure to determine the presence or absence of hyper-immune anti-A or anti-B follows:

To one part of the mother's serum add two parts of Witebsky's AB Group Specific Substance and one part physiologic saline. Make serial two-fold dilutions in saline of this 1:4 dilution of mother's serum and measure 0.1 ml. of each dilution as made into a 10 x 75 mm. tube correspondingly marked. To each tube add 0.1 ml. of a 2% saline suspension of Group A (or B), Rh negative cells. Make a second series of dilutions, starting with the 1:4 dilution of mother's serum, using Group AB serum in every instance in place of the saline. Allow both titrations to stand at room temperature for one hour. Centrifuge at 1000 r.p.m. for one minute and examine for agglutination. The amount of AB substance to be added is an arbitrary figure, but should be the minimal amount required to neutralize nearly completely the natural antibodies, which are demonstrable in saline. Ideally, after neutralization, there should remain in the saline 1:4 dilution enough antibody to give about a ++ agglutination of the test cell, i.e., grossly visible by small clumps of cells.

Interpretation: If the serum is completely neutralized, i.e., the cells are not agglutinated in either the saline or AB serum dilutions, there are probably no hyper-immune antibodies present in the mother's serum. If, however, there is an appreciably higher titer in the serum dilutions than in the saline dilutions, a 2-3 tube difference, hyper-immune antibodies are present. The presence of anti-A antibodies in a Group A infant (or anti-B in a Group B infant) aids in the diagnosis of erythroblastosis due to major blood group incompatibility.

Jaundice is the most important clinical finding in all of these infants. It is not present at birth but develops in the first 24 hours of life. An indirect bilirubin of 10 or more mgm./100 ml. during the first 24-hour period and 15 or more mgm./100 ml. in

the next 24 hours is practically diagnostic of the disease, assuming that infection and enclosed hemorrhage (in the brain, pulmonary or abdominal cavities) which also cause jaundice are absent.

After birth, most babies are unable to excrete bilirubin rapidly enough during the first 48 hours of life. Therefore, it accumulates; serum bilirubin levels of 30 to 40 mgm./100 ml. may develop in infants with erythroblastosis fetalis. Bilirubin, or something closely allied to it, causes the brain damage known as kernicterus; consequently it must be kept from rising too fast and too high. Therefore, exchange transfusion is the only rational mode of treatment for the first 2 to 3 days. Compatible, fresh (usually Rh negative) whole blood, preferably from women donors, is used at the Children's Hospital. As many as two or three exchange transfusions have been given at 12-hour intervals, in order to keep the bilirubin below 20 mgm./100 ml. which seems to be a critical level.

The brain lesion known as kernicterus is one of the principal complications of erythroblastosis fetalis. The recovery rate from kernicterus has increased from 75 per cent (8 years ago) to 95 per cent at the Children's Hospital in Boston, and to nearly 100 per cent for the past 5 years at the Richardson House and Boston Lying-In Hospital. The reason for this is that the mothers who are to be delivered at these latter two hospitals are followed very closely during pregnancy.

When the obstetrician is ready to deliver a mother who has a titer, he notifies the Blood Grouping Laboratory and one of our physicians is on hand at the delivery. The baby's blood is tested immediately, the Blood Bank having been notified in advance, has fresh compatible Rh negative blood ready. If the tests indicate it, exchange transfusion is begun less than an hour after birth. Unfortunately, at the Children's Hospital some babies are not referred for admission until they have been jaundiced for 24 or more hours, and in some of these cases, brain damage has taken place before an exchange transfusion can be started.

Kernicterus tends to have a familial character, i.e., the liability to kernicterus is greatest in families in which there is already one case, and least in families with several erythroblastotic infants but without kernicterus. A poorer prognosis in erythroblastosis fetalis may be expected in cases with a history of a previous affected infant than if there is no such history. The likelihood of a stillborn is greater in the cases of mothers with a high titer who have had previous infants stillborn because of erythroblastosis fetalis, but the prognosis is never hopeless.

A transfusion of Rh positive blood to a woman who is Rh negative is a much more effective means of iso-immunization

than pregnancy. Nearly 100 per cent of Rh negative people can be immunized by transfusions as compared with 5 per cent of Rh negative women with Rh positive husbands. But, once sensitized, the future pregnancies are certainly no worse than if sensitization resulted from pregnancy, and there is a much greater chance that the mother will have an Rh negative baby (since more than 50 per cent of husbands will be heterozygous).

Rh negative persons vary greatly in their responses to injections of Rh positive blood. About 40 per cent develop Rh antibodies after two intravenous injections of Rh positive blood given at an interval greater than a week, but a few individuals fail to show any evidence of sensitization even after repeated injections. The amount of the transfused blood is not the controlling factor. As little as 0.05 ml. of Rh positive blood is sufficient to start iso-immunization, though larger amounts may be needed. Certainly as little as 5 to 10 ml. is a good immunizing dose.

A possible error in testing the blood of a transfused patient may be mentioned here. If the blood of an Rh negative person is tested within a few hours or even a day or two after an Rh positive transfusion, there may be an appreciable number of Rh positive red cells present, even when the patient has shown clinical signs of hemolysis. Therefore, unless care is exercised, the patient may appear to be Rh positive. More exact typing and microscopic examination will show that the picture is one of a small proportion of agglutinated cells (the donor's cells) with a preponderance of unagglutinated Rh negative cells (the patient's cells).

In 1946, Stratton described a third alternative to D and d and called it D^u. Unfortunately, the D^u variant is not a single specific blood group substance that can be identified by a specific typing serum, but rather a large group of variants, very closely related to D (Rh_o) and distinguishable from D only by quantitative methods. Red cells of the D^ud specificity were recognized by being agglutinated by some anti-D sera but not by others. The different grades of the D^u antigen have been classified as (1) cells of the "higher" grade which are agglutinated by certain anti-D sera and (2) cells of the "lowest" grade distinguishable from the Cde and cdE by a positive Coombs' test only with an incomplete anti-D serum. The incidence of D^u among supposedly

	rh′	rh″	r′r″
D^u —	11	5	0
not D^u —	23	41	1
	34	46	1

Therefore about half of the apparent rh′ individuals are actually CD^ue or R_1^u.

The D^u variant is antigenic, i.e., it can cause the production of

ordinary anti-D in an Rh_0 (D) negative recipient and may also cause a fatal transfusion reaction if the recipient is already sensitized to D. The routine use of at least **two different** anti-D sera is recommended to increase the probability of identification of this antigen. The best safeguard is the indirect Coombs' test in conjunction with a high-titered incomplete anti-D serum to screen all Rh negative (rr) bank bloods.

Race et al. found that different samples of $D^u d$ red cells usually did not give identical reactions when tested against a battery of agglutinating and incomplete anti-D sera. On the other hand, with one exception, blood of **related** $D^u d$ persons did give identical reactions making it practically certain that even these fine distinctions are genetically determined.

Sensitization by transfusion does not lead necessarily to all babies having erythroblastosis fetalis because:

1. The father may be Rh negative (rr)—in which case the mother never would have been sensitized unless she had received the incompatible blood. All babies will be Rh negative and consequently will not have the disease, in spite of the anti-Rh antibodies in the mother's serum.—or
2. The father may be heterozygous in which case 50 per cent of his children will be Rh negative (rr).

When a diagnosis of erythroblastosis fetalis is anticipated in a newborn infant on the evidence of serologic tests done on the mother's serum prior to delivery, the first most important test to be done on the baby's blood, immediately after birth, is the direct Coombs' test. If this test is positive the diagnosis can be made with certainty. If anti-D is present in the mother's serum and the direct Coombs' test is positive, it can be assumed that the disease is caused by anti-D.

Other laboratory tests arranged in approximate order of their importance are (2) the blood group and Rh of the baby,* (3) bilirubin level,** (4) hemoglobin—a low hemoglobin at birth or in the first few days of life strongly suggests erythroblastosis fetalis. However, a high hemoglobin and red blood cell count during the same interval do not rule out erythroblastosis and it is dangerous to think of a high hemoglobin as being a favorable sign. (5) Reticulocyte and nucleated red count—an elevation in the number of these indicates increased activity of the bone marrow to compensate for the destruction of the baby's red cells. However, any anoxia will do the same, e.g. asphyxia during labor, congenital heart lesion or pulmonary atelectasis.

With the diagnosis of erythroblastosis fetalis established on a clinical basis, the most valuable laboratory tests for the treatment and prognosis of the disease in order of their importance are:

1. **Hemoglobin** (if very low). The age of the baby is important.

If the hemoglobin is low at birth, the disease is more severe.

2. **Bilirubin.** If the hemoglobin is not very low at birth, this is the most important test. A baby with a high hemoglobin will have more pigment bilirubin in his serum if blood destruction is taking place than the baby with a low hemoglobin who is destroying his red cells at the same rate.

3. **Reticulocyte and Nucleated Red Cell Counts:** If these are high, it indicates rapid cell production and the usual inference is that the blood is being destroyed rapidly, unless there has been a hemorrhage.

4. **Maternal Antibody Titer:** As many as 30 per cent of erythroblastotic babies whose mothers have titers of less than 1:16 may have no illness at all. When the maternal titer is 1:64 or higher, 30 to 50 per cent of the babies will be stillborn and there is an increased risk of severe disease or of kernicterus in a liveborn infant. But the first affected baby frequently has the disease to a lesser degree than subsequent ones.

If a pregnant woman is Rh negative (D negative), her blood must be tested to determine whether or not she has been sensitized to this factor. The Rh typing and the test for sensitization can be done at the same time, preferably at about 32 weeks gestation—earlier if there is a history of erythroblastosis fetalis in a previous baby or if the mother has received a blood transfusion or intramuscular injection of blood even in early childhood. If no antibodies are found at this time, the test should be repeated three or four weeks before term.

The routine screen tests for Rh antibodies used in the Blood Grouping Laboratory consist of testing the mother's serum against a panel of four Group O bloods which have been treated with a 0.1 per cent trypsin solution. These cells are of known and varied Rh types and are selected to contain among them all the known blood group antigens except A and B. The Trypsin-Modified cell technic has been described by Miss Taylor.

The second test we do is the Diamond-Abelson Slide Test. Two drops of the mother's serum are mixed with three drops of whole blood, preferably from a Group O, R_1R_2 (CDe/cDE) person. (We have observed that if the test cell is used the same day it is drawn, frequently non-specific granulation occurs which can be confused with true agglutination. For best results the oxalated blood should not be less than 18 hours and not more than 96 hours old.) The cells and serum are mixed on a microscope slide by means of a wooden applicator stick and spread out in a rectangle about 2" x 1". The slide is then placed on a

* The red cells of a baby who has a positive direct Coombs' test may be so heavily coated with a blocking antibody that they are not agglutinated by ordinary anti-D sera and the baby may be erroneously classified as Rh negative.

** Hsia, D. Y., Hsia, H. H., and Gellis, S. S., A micromethod for serum bilirubin. J. Lab. and Clin. Med., 40:610, 1952.

warm viewing box and rocked back and forth occasionally and observed for agglutination. It is important to remember that you are *not* dealing with potent typing reagents but with a completely unknown serum, possibly a very weak one, which may react very slowly with the known Rh positive test cell and give rather poor agglutination in the end. If the serum contains anti-Rh antibodies, the cells will be agglutinated. A control should be run in parallel, using 2 drops of a normal serum and 3 drops of the oxalated blood.

We also do an indirect Coombs' test against one Group O (D positive, K positive, Fy^a positive) blood routinely. If any of these tests is positive, the mother's serum is then tested against another panel of four completely typed Group O, Rh negative cells in order to try to discover the presence or absence of additional antibodies other than Rh, such as anti-Kell, anti-Duffy, anti-Kidd, etc. These cells are made up in a 2 per cent saline suspension, 2 drops of each saline suspension are mixed with 2 drops of the mother's serum in a 10 x 175 mm. tube and incubated at 37° C. for one hour, centrifuged at 500 r.p.m. for one minute and examined. If there is no gross agglutination visible when the tubes are shaken gently to dislodge the cell button, the tubes are centrifuged again, the saline removed, and 2 drops of 25 per cent albumin are added. The cells are resuspended in the albumin, allowed to stand at room temperature for 10 minutes, centrifuged at 1500 r.p.m. for two minutes, and examined again. If no agglutination is observed at this point, the cells are washed four times with large volumes of fresh saline and then anti-human serum is added.

1.1% Positive reactions non-specific for known Blood Group Antigens.

Negative results against the D negative cells indicate that anti-D is the only anti-body present; positive results with the other cells and negative with the R_1R_1 cell indicate that hr′ (c) is the antibody present; a positive result by the indirect Coombs' test only against a particular cell indicates that one of the rarer antibodies, S, Kell, etc., is present.

The next step is to determine the titer of the antibody present by making serial fourfold dilutions of the patient's serum in saline, and testing each dilution against the appropriate Group O cell. The tubes are incubated at 37° C. for one hour, centrifuged at 500 r.p.m. for one minute, and examined for agglutination. Then the tubes are centrifuged again, the saline removed and 25 per cent albumin added, as described above. The endpoint is the last tube in which small but definite agglutinates are visible.

If the mother's serum does contain an antibody, it is well, if at all possible, to determine the zygosity of the father for the

particular antigen involved. If the father is heterozygous for the antigen and if there is no apparent change in the mother's titer during pregnancy, there is a good possibility that the baby is the same type as the mother and will not have erythroblastosis fetalis.

ANTIBODY SCREENING PANEL

	C.P.	P.M.	W.C	F.R.	H.T.	M.L.
C	+	0	0	+	0	0
C^w	+	0	0	0	0	0
D	+	+	0	0	0	0
E	0	+	+	0	0	0
c	+	+	+	+	+	+
e	+	+	+	+	+	+
M	+	+	0	+	+	0
N	+	0	+	+	+	+
S	0	+	0	+	+	+
P	+	0	0	+	0	+
K	0	0	0	+	0	+
k	+	+	+	+	+	+
Le^a	0	0	+	0	0	+
Le^b	+	+	0	+	+	0
Lu^a	0	+	0	+	+	0
Fy^a	+	+	0	+	0	+
Jk^a	+	+	+	+	+	+

TABLE 3

Summary

Erythroblastosis fetalis is a disease of infancy resulting from blood group incompatibility between parents. It is not a very common disease—it occurs approximately in 1 in 100 pregnancies. About 80 per cent of all cases of erythroblastosis result from anti-D incompatibility, 10 to 15 per cent on the basis of anti-A or B and about 5 per cent from anti-Kell, S, Kidd, etc. Infants who survive the hazards of intra-uterine hemolysis and are born alive are exposed to the possibility of kernicterus. By exchange transfusion the recovery rate from this disease has been increased at the Children's Medical Center, Boston, from 75 per cent (8 years ago) to 95 per cent and it has increased further (almost to 100 per cent) in the past 5 years at the Boston Lying-In Hospital. Jaundice is the most important symptom of the disease to be watched for in order to avoid kernicterus and bilirubin is the most important measurement in the first few days of life. An indirect bilirubin of over 20 mgm./100 ml. seems to be the critical level in the first two to three days. Exchange transfusion, using compatible, fresh, Rh negative blood is the only certain way of reducing this level. An injection of Rh positive blood is a much more effective means of sensitizing an Rh negative woman than pregnancy alone. The amount transfused is not too important. As little as 0.5 ml. of Rh positive blood may be sufficient to start iso-immunization.

TABLE 4

The following table of Blood Group antigens (except A, B, and D) is arranged in approximate order of their antigenic potency, their frequency, and the usual method of identification.

HEMOLYTIC TRANSFUSION REACTION			ERYTHROBLASTOSIS FETALIS				NO CLINICAL IMPORTANCE		
Antigen	Frequency	Best Medium	Antigen		Frequency	Best Medium	Antigen	Frequency	Best Medium
K	10.0%	ICT	K	5 Percent of all cases	10.0%	ICT	N	71.0%	Saline @ Rm. T
k	99.7%	Saline	C		70.0%	Alb.	P	70.0%	Saline @ 4°C.
C	70.0%	Alb.	C^w		1.0%	Alb.	Lu^a	10.0%	Saline @ Rm. T
C^w	1.0%	Alb.	c		80.0%	Saline Alb. ICT	Le^a	75.0%	Saline @ 14°C
c	80.0%	Saline Alb. ICT	E		30.0%	Saline Alb.	Le^b	70.0%	Saline @ 14°C.
E	30.0%	Saline Alb.	k		99.7%	Saline			
e		Saline	M		79.0%	Saline @ Rm. T			
M	79.0%	Saline @ Rm. T	S		55.0%	Saline ICT			
S	55.0%	Saline ICT	JK^a		77.0%	ICT			
Fy^a	65.0%	ICT	Fy^a		65.0%	ICT			

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This lists only some of the basic references

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THE TRYPSIN-MODIFIED CELL TECHNIQUE FOR DETECTION OF RH ANTIBODIES

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I. Introduction

Rh antibodies may be classified according to the manner in which they react as well as according to their specificity. In the following discussion of Rh antibodies and their detection, reference will be made only to Anti-D (Rh_o) since other antibodies may react similarly. Only those antibodies whose mode of action can be determined by serologic tests in a routine laboratory will be considered.

Isoagglutinins for the Rh factor do not occur naturally, but are the result of active isoimmunization, either by pregnancy or blood transfusion. Antibodies exist in what may be called a spectrum of different forms and may be identified according to the manner in which they react as shown in Table I.

TABLE I. MODES OF ACTION OF ANTIBODIES

Aggl. in Saline	Aggl. in Albumin	Blocking in Saline	Aggl. with Coombs Sera	Type of Antibody
+	+	..	..	"Saline Active"
—	+	—	+	"Albumin Active"
—	+	+	+	"Albumin Active"
—	—	—	+	"Coombs Active"

"Saline Active" sera are those which contain antibodies which will agglutinate red cells in saline suspensions. This type of antibody is known as saline, early-immune, bivalent, complete antibody or agglutinin.

A serum which contains an antibody which is capable of agglutinating red cells suspended in a protein or colloid media, such as serum, albumin, photo-engraving glue, polyvinylpyrrolidone, etc., but which will not agglutinate cells suspended in saline, is an "albumin active serum." Such a serum will also give a positive indirect Coombs test after incubation with saline suspended cells.

One type of "albumin active" serum can specifically "coat" saline suspended Rh-positive cells. Such "coated" cells are then

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unable to react with the "saline active" type of Anti-Rh testing sera.[1] This is known as the "blocking" reaction.[2] It is this type of antibody that is responsible for prozones in saline titrations.

An occasional serum may, in addition to the "albumin active" antibody, also contain a type of antibody which will "block" in protein media as well as in saline and thus prevent the reaction of such "blocked" cells with another "albumin active" type of anti-Rh testing serum. This type of antibody in a serum results in a prozone reaction when titers are performed using a protein medium as diluent.

"Albumin active" antibodies are known as incomplete, hyperimmune, univalent, or blocking antibodies or glutinins. These "albumin active" antibodies which specifically "coat" but do not "block" are known as cryptagglutinoids.[3,4] It would seem preferable to reserve the term "blocking antibodies" for those antibodies which actually specifically "coat" and "block" rather than use it as a general term to cover all "albumin active" types of antibody.

A serum which contains the type of antibody which is demonstrable only by the indirect Coombs technique will be referred to as "Coombs active." The "albumin-" and "Coombs-active" types of antibody are considered to be the etiological agents in Erythroblastosis. All of these varieties of antibody are specific in relation to the D antigen; one, all, or various combinations of them may be present in the same serum. The differences in antibodies has resulted in a variety of techniques to demonstrate the presence of an antibody in a serum.

The more common means of demonstrating the presence of Rh antibodies are illustrated in Table II. (Details of these tests may be found in the literature, 5, 6, 7, 8, 9 and 10).

The "saline active" type of antibody is detected with normal cells suspended in saline, the serum being tested is diluted with saline for titrations and a period of one hour incubation is required. Three methods are available for the detection of "albumin active" type of antibody. One method involves the use of normal cells suspended in saline, serum diluted with saline, a period of incubation, then replacement of the saline with a colloid such as albumin, photoengraving glue, serum-albumin mixtures, serum, or polyvinylpyrrolidone. In the second method albumin is used both as cell suspending medium and as diluent. The third method involves the use of trypsin-modified cells, using saline as the cell suspending medium and as diluent. "Albumin active" as well as "Coombs active" antibody may be demonstrated by the use of the indirect Coombs test.

We have found the trypsin-modified cell method of detecting antibodies to be extremely useful in a routine Rh laboratory.[11,12]

Trypsin-modified cells are agglutinated by all but the "Coombs active" type of antibody. The use of this method simplifies the problem of detection of Rh antibodies, and makes it possible for even the smallest of laboratories to offer an Rh service. The method is accurate, sensitive and simple. It can be made specific by carefully controlling the conditions under which it is used.

TABLE II. METHODS OF DETECTING ANTIBODY

Type of Antibody	"Saline Active"	Albumin Active			"Coombs Active"
Cells	N	N	N	TM	N
Suspension Media	S	S	15% Alb.	S	S
Diluent	S	S	20% Alb.	S	S
Incubation	1 hour 37°C	½ hour 37°C	None	5 Min.	1 hour 37°C
CENTRIFUGE					
Aggl'tion	+	—	+	+	—
	RECENTRIFUGE AND DECANT				Wash 3x's saline
Add	..	Colloid	..	..	Coombs Sera
Aggl'tion	..	+	..	..	+

N — Normal Cells
S — Saline
TM — Trypsin Modified Cells

Colloid — Any protein media such as albumin, photo-engraving glue, serum, etc., also includes polyvinylpyrrolidone.

II. Method for the Preparation of Trypsin-Modified Cells

Several factors such as time interval, pH, temperature, enzyme strength and presence of trypsin inhibitor in sera effect the degree of enzyme modification of red cells. There are numerous variations of these conditions which could cause the desired modification of red cells. We have selected a set of conditions which we have found give satisfactory results. These include a time interval of 10 minutes, because it is convenient and easily measured; a temperature of 37° C because it is available; a pH of 7.1 for the final cell-trypsin-buffered saline system, because it is physiologic for the cell; the neutralization of trypsin inhibitors by dilution. The concentration of enzyme, 0.1%, was chosen arbitrarily.

The method for trypsin modification of red cells is as follows:

0.25 cc. Fresh whole blood, taken in 1/10th its volume of ACD Solution
4.5 cc. Phosphate buffered saline, pH 7.2
0.5 cc. 1% Trypsin Solution
Incubate **exactly** 10 minutes at 37° C.
Centrifuge.
Decant supernatant.
Resuspend cells in 5 cc. normal saline.

III. Standardization of the Method

The reasons for the choice of this particular combination of factors have been given elsewhere.[12] The most pertinent of these findings may be summarized as follows:

A. Concentration of Enzyme Used to Modify Cells.

By treating cells with 0.1, 0.2, 0.4 and 0.8% trypsin it was found that while increasing the percentage of trypsin increased the number of sera giving specific reactions for anti-D, it also increased the number of non-specific reactions. The increase in the number of non-specific reactions was far greater than the increase in specific positive reactions. The 0.1% trypsin concentration was selected because it gave efficient sensitivity with a minimum of non-specificity.

B. Potency of Crude Trypsin

Different lots of Crude Trypsin (Difco 1:250 Trypsin) were found to vary in potency. It was found that only when different lots of trypsin were assayed for proteolytic activity by gelatin titrations, and then diluted to comparable proteolytic strength did they give identical degrees of modification of red cells. However, once the potency of the enzyme preparation had been determined the standardization procedure did not have to be repeated oftener than once every six months or a year. Fortunately, the Difco Laboratories now have available a lypholyzed trypsin and buffer which is pre-assayed so that results are consistent from lot to lot, and the need for restandardization is eliminated.

C. Effect of Cold Agglutinins Upon Trypsin-Modified Cells

If red cells are overtreated with trypsin they will be agglutinated by a great many cold agglutinins at room temperature. These reactions can be reduced to a minimum by following exactly the procedure for trypsinization that has been outlined. Also allowing the trypsin-modified cells and serum to stand together for 5 minutes before centrifuging will help to reduce reactions with cold agglutinins even further. Some cold agglutinins are also inhibited by the phosphate ion in minute amounts; therefore, cells are not washed after trypsin-modification. There will still be a small number of non-specific agglutinations with trypsin-modified cells at room temperature, as there will be with other methods. For this reason, it is necessary to include a trypsin-modified Rh-negative control cell with the trypsin method, just as it is necessary to include an Rh-negative cell control with the other methods.

IV. Screening Test for Rh Antibodies

In our Rh Laboratory at The Children's Hospital, Columbus, Ohio, blood from Rh-negative pregnant women is screen-tested for Rh-antibodies. This screening is a 3-tube test which checks for "saline" and "albumin active" antibodies. Two drops of undiluted serum are placed in each tube; to the first is added one drop of saline suspended Rh-positive (O CcDEe) cells; to the second, one drop of trypsin-modified Rh-positive cells; and to the third, one drop of trypsin-modified Rh-negative (O cde) cells. After 5 minutes the tubes are centrifuged at 1500 rpm for 2 minutes and read for agglutination. The first tube is recentrifuged and decanted, and 2 drops of either 10% photoengraving glue, PVP or 20% albumin may be added. After 5 minutes the tube is again centrifuged and read for agglutination. A diagram of the screening test may be found in Table III.

TABLE III. SCREENING TEST FOR ANTIBODIES
2 drops of serum being tested in each of 3 tubes
1 drop of cells as follows:

Tube No. 1 N Rh + Cells	Tube No. 2 TM Rh + Cells	Tube No. 3 TM Rh — Cells	Recentrifuge Tube No. 1. Decant. Add Colliod. Recentrifuge.	Aggl. in Tube No. 1	Interpretation
Centrifuge after 5 minutes					
—	—	—		—	No Antibody
—	+	+		—	Antibody other than Rh
+	+	+		+	Rh Antibody Present
—	+	+		+	Rh Antibody Present

N..........Normal Rh-Positive Cells in Saline.
TM Rh +..Trypsin Modified Rh-Positive Cells.
TM Rh —..Trypsin Modified Rh-Negative Cells.

From September 1947 to January 1953, 4572 Rh-negative women have been screen tested with trypsin modified cells and glue, with the following results:

3553	Negative
69	Positive Trypsin Only
890	Positive Trypsin and Glue
61	Positive due to other than blood group antigens
4573	Total Tests

1.1% positive reactions non-specific for known Blood Group Antigens.

V. Titrations of Rh Antibody

Titrations of serum are made with calibrated syringe automatic pipettes. The use of these pipettes eliminates much of the variation involved in titration techniques and provides more consistent results. While the method admittedly allows a slight error due to "carry-over" this constant error is far offset by the

advantages of speed and consistency.

Tuberculin syringes (1 cc.) are fitted with blunted needles and a device cut from sheet metal which clamps around the barrel and extends over the top of the syringe to limit its intake of fluid. A set of 2 tuberculin syringes are calibrated, using hemoglobin solution and a spectrophotometer, so that one pipette will contain exactly the amount of fluid that the other pipette delivers.

Titrations are performed by using the "delivery pipette" to deliver 0.2 cc. of the diluent to all tubes in the titration, and 0.2 cc. of the serum to the first tube. The "contain pipette" is used to mix the contents of the first tube and to transfer the mixture to the next tube. The process is repeated until the transfer has been completed through each succeeding tube of the titration. A diagram of the pipettes is shown in Figure 1.

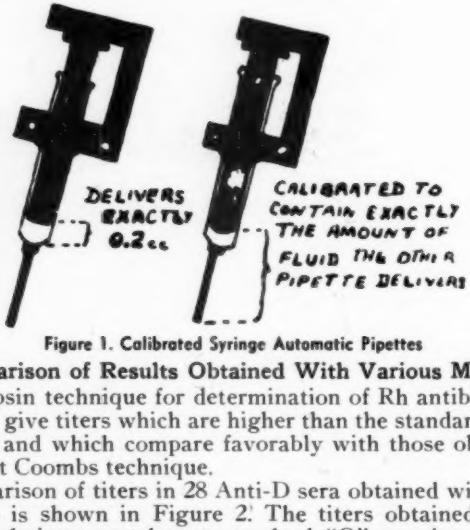

Figure 1. Calibrated Syringe Automatic Pipettes

VI. Comparison of Results Obtained With Various Methods

The trypsin technique for determination of Rh antibodies will, in general, give titers which are higher than the standard albumin technique, and which compare favorably with those obtained by the indirect Coombs technique.

A comparison of titers in 28 Anti-D sera obtained with various techniques is shown in Figure 2. The titers obtained with the albumin technique are taken as standard, "O" meaning the titer is the same as that obtained with albumin. The numbers indicate the number of tubes that the end-point of the titer was greater or less than that obtained with albumin. The length of the bar represents the number of sera giving that reaction.

Figure 3 shows the results obtained in another series of specimens, comparing the titers obtained with trypsin-modified cells and PVP, using trypsin-modified cells as base. The titers obtained with PVP are somewhat higher than those obtained with trypsin-modified cells; however, the end-points of the titers are

not as clear cut as those obtained with trypsin-modified cells and are easily over-read. The end-point with PVP often depends upon the amount of agitation used in dislodging the cell button.

When comparing titers it is necessary that all the methods being compared be run on a serum at the same time. Many conditions, such as room temperature, age of cells, intrinsic error in the methods, etc., effect the end-point obtained. For example, in Figure 4 are shown the end-points of titers obtained when this series of weekly specimens from one individual were run on the day that they were drawn, and the end-points obtained when the titers were run simultaneously.

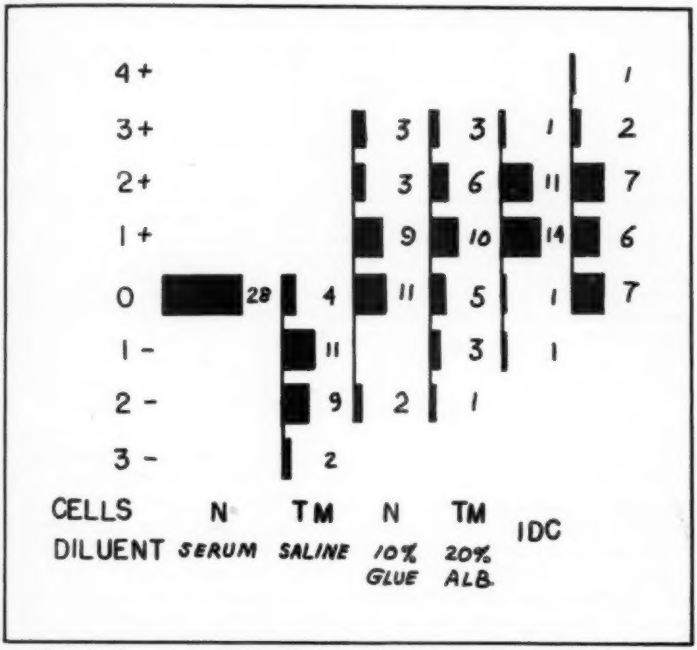

Figure 2. Comparison of Titers Obtained in 28 Sera Using Various Techniques, Albumin Taken as Standard.

The numbers in the column on the left indicate the number of tubes that the end-point of the titer was greater or less than that obtained with albumin, while "O" indicates that the titer is the same as that obtained with albumin.

The figures at the end of the bars indicate the number of sera giving that reaction.

N = Normal cells suspended in saline
TM = Trypsin modified cells suspended in saline
Alb. = Bovine albumin
IDC = Indirect Coombs Test

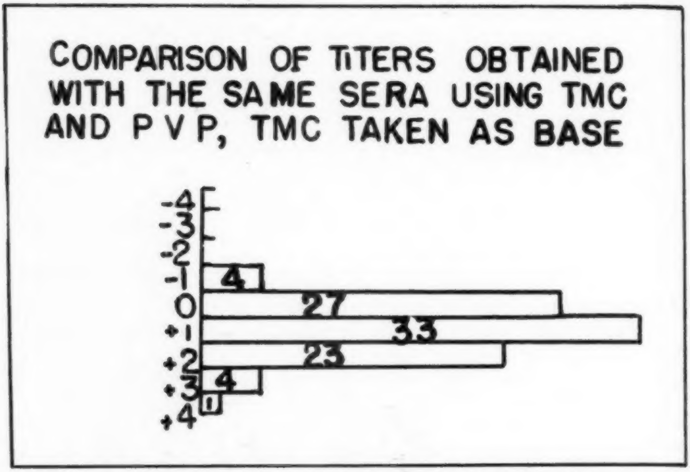

Figure 3. Comparison of Titers Obtained With Trypsin-Modified Cells and Polyvinylpyrrolidone Techniques.

The numbers in the column on the left indicate the number of tubes that the end-point of the titer with PVP was greater or less than that obtained with trypsin-modified cells. "O" indicates the titers with PVP that were the same as that obtained with trypsin-modified cells. The figures on the bar indicate the number of sera giving that reaction.

VII. Discussion

A. Advantages of the Trypsin-Modified Cell Technique

1. *Agglutination is easy to read.* Since saline is used as the cell suspending medium, and as diluent for serum, the centrifuged button of cells is more easily dislodged from the bottom of the tube by gentle agitation than in the case of viscid suspensions such as albumin, albumin-serum mixtures, or PVP. This greatly increases the ease of reading the tests and the sharpness of the end-point in serum titrations.

2. *Agglutination is immediate.* In common with the albumin tube technique the agglutination is complete on immediate centrifugation. However, a waiting period of five minutes is recommended when serum of unknown antibody contest is being tested, since this may eliminate confusion with an occasional cold agglutinin.

3. *Prozones are abolished.* With the trypsin-modified cell technique undiluted sera may be used for screening tests with confidence that anti-Rh antibodies will not be missed because of prozones.

4. *High index of sensitivity.* Appropriately trypsin-modified cells can detect smaller amounts of antibody than any other method except the indirect Coombs test, and the use of PVP.

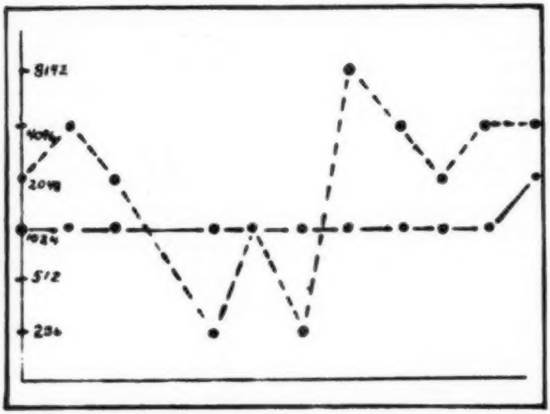

Figure 4. Titers Obtained With Trypsin-Modified Cell Technique On Specimens Obtained at Weekly Intervals From One Individual.
Straight Line = Titers obtained when the tests were performed simultaneously on all specimens.
Broken Line = Titers obtained when the tests were performed individually on the same day that the specimens were drawn.

5. *Materials are inexpensive.* Compared to the cost of bovine albumin and Coombs serum the cost of crude trypsin is negligible, and once it is standardized it may be used over a long period of time. The use of the newer lypholyzed trypsin and buffer for hemagglutinations eliminates the need for standardization, and makes the procedure available to the smaller laboratories.

B. Disadvantages of the Trypsin-Modified Cell Technique

1. *Interference of cold agglutinins.* The main disadvantage of the trypsin technique is that non-specific reactions can occur as the result of interference of cold agglutinins. This can be reduced to a minimum by carefully observing the conditions that have been established for the use of the test. These conditions include:

a. Control of the potency of trypsin and the concentration of enzyme used to modify cells.
b. Observation of the exact conditions of time, temperature and pH used to modify cells.
c. The use of fresh cells, and not washing cells before or after trypsinization.
d. The use of a test cell panel which includes both trypsinized Rh-positive and trypsinized Rh-negative cells.

2. *Failure to demonstrate "Coombs Active" antibodies.* Another disadvantage is that the method does not demonstrate those antibodies which react only with the indirect Coombs test, such as found in a

few anti-Rh sera, in most anti-Kell, in all anti-Duffy, and in several other antibodies due to other rare blood group antigens. However, the necessity to test further for these is usually apparent from the clinical aspect of the patient, and also, in the case of the newborn, from a positive direct Coombs reaction.

VIII. Conclusion

In our laboratory, we have found that the advantages far outweigh the disadvantages, and that the use of red cells modified by trypsin under standard, carefully controlled conditions offers an improvement over previous methods for Rh antibody detection.

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ADDENDUM

Details of Additional Methods Mentioned in The Text

I. Gelatin Assay of Trypsin Activity

Dissolve 4 grams of Bacto-Gelatin (Difco) in 100 cc. buffered saline at 37° C.

Dilute stock 1% trypsin solution with 9 parts of buffered saline.

Serially dilute 0.2 cc. of this concentration in 0.2 cc. amounts of buffered saline through a series of 8 tubes.

Add to each tube 1.0 cc. of the warm gelatin solution and mix tubes thoroughly, avoiding foaming.

Incubate tubes in a waterbath at 37° for exactly 30 minutes, then place in the refrigerator at 4°C for at least 12 hours.

Read immediately as tubes are taken out of the refrigerator, the end-point is that tube containing the least concentration of trypsin which liquefies the gelatin sufficiently to prevent inversion of the tube without spillage.

A concentration of trypsin which is 16 times as strong as that of the end-point tube is comparable to that used by us and found to give both satisfactory specificity and sensitivity.

II. Buffered Saline

Make a stock phosphate buffer solution pH 7.3 by mixing 7.7 cc. of 0.07 molar Na_2HPO_4 and 2.3 cc. of 0.07 molar KH_2PO_4.

Mix one volume of the buffer with 9 volumes of 0.85% NaCl solution.

The actual pH of the buffered saline is about 7.2 as determined by glass electrode.

III. Calibration of Automatic Pipettes.

A Coleman spectrophotometer, filter Pc-4, wave length 550, was used for calibration, but any photometric equipment used for hemoglobin determinations would be satisfactory.

Lake 6 drops of whole oxalated blood in 5 cc. distilled water.

Deliver 0.2 cc. of laked blood into 5 cc. of distilled water with a serological pipette and read the optical density, using distilled water as the blank.

Set the "Delivery Pipette" at approximately 0.2 cc. Deliver the quantity of laked blood it will contain into 5 cc of water, and read the optical density.

Adjust the stop on the syringe and repeat the procedure until it gives the same reading as the serological pipette.

Set the "Contain Pipette" so that when its contents are washed out into 5 cc. of water the optical density reading is the same as that obtained with the "Delivery Pipette."

Cement the metal stops in place.

INDIRECT COOMB'S COMPATIBILITY

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More than 3,000,000 transfusions are given each year in this country and the figure is mounting year by year. This increased use of blood has created many new problems in the routine operation of a blood bank. More and more patients are receiving multiple blood transfusions during major surgery and for the control of the anemias, with the result that many patients who come to us for blood transfusion today have had transfusions previously. One of the serious sequalae of multiple and repeated transfusions is that many patients become immunized to the Rh-Hr factors or to one or another of the newly discovered blood groups, some of which may have been unknown at the time of their transfusions. The problem facing the blood bank today is to determine how these patients may be transfused with safety.

For many years the compatibility technique has been done in saline, both major and minor compatibilities being done or major compatibilities alone, but the only medium used was saline. Until the discovery of blocking antibodies, this served the purpose, since one determined that the patient's serum was compatible with the cells of the blood to be given. With the discovery of the so-called blocking antibodies, however, saline suspensions were no longer satisfactory, for these new antibodies do not agglutinate saline-suspended cells. Many of the newer antibodies (Duffy, Kell, Kidd) were also found to be of this same type that could not be detected in saline, and several ways of improving the compatibility were suggested. The problem in brief was to find a compatibility which would detect all the known antibodies and at the same time be practical for use in the laboratory. Probably the most popular technique used at the present time is the "high protein media" (albumin, AB serum). At the New York Hospital we have not used such media extensively, for though the technique is simple, it is not entirely satisfactory, and its disadvantages, in our opinion, outweigh the advantages. These are chiefly that rouleaux formation may falsely simulate incompatibility, and also, it fails to detect some important antibodies. P.V.P. has also been tried out, but this too seems to have many disadvantages.

Incompatibility in the ABO system is quite easy to detect in saline. If there has been error in the grouping of the blood of either the patient or the donor, the routine saline compatibility should detect it readily. However, when one is dealing with an

antibody of the blocking type, the saline medium will not serve the purpose. Furthermore, since many of these antibodies are not detected in albumin or in P.V.P., and even the trypsin techniques miss antibodies like Kell and Duffy, the only known way at the moment to detect with certainty these unusual antibodies is by means of the Indirect Coombs technique.

The following case demonstrates what can happen if the Indirect Coombs compatibility is not performed.

Fig. 1 (Case No. 1)

Case # 1

A.D. Physician, Age—36
Bleeding peptic ulcer

Pt.—Group O, Rh pos.

Donor—Group O, Rh pos.

Pt. serum X Donor cells (sal.) .... Compatible

Pt. serum X Donor cells (alb.) .... Compatible

Pt. serum X Standard cells (tryp.) .... Compatible

Pt. serum X Donor cells (tryp.) .... Compatible

Hemolytic transfusion reaction—
Cyanosis, Drop in B.P., Hb-uria, Jaundice.
Recovered.

Cross-matching rechecking .... Compatible

Pt. serum X Donor cells (I.C.) .... + + + +

Pt. serum X Cell Panel (I.C.) .... anti Fy^a

Pt. cells .... Fy^a—

Donor cells .... Fy^a+

Pt. serum .... anti Fy^a 1:64

At the New York Hospital it is our routine practice to test the serum of every patient and every donor for the presence of antibodies by several different techniques. Every serum is tested first against a trypsinated standard cell. We are fortunate to have at our disposal a rather unusual standard cell which we have used in the trypsin and Coombs techniques.

Fig. 2 (Standard Cell R.J.)

STANDARD CELL R.J. —GROUP O

C +	M +	Duffy (Fy^a) +
c +	N —	Kell (K) +
D +	S +	Cellano (k) +
E +	s —	Kidd (JK^a) +
e +	P +	Lewis (a) —
	Lu +	Lewis (b) +

Every ante-natal patient both Rh positive and Rh negative is also tested for the presence of antibodies, using the trypsinated cell. If the obstetrical patient is Rh negative, her serum is further tested with the Indirect Coombs technique.

For crossmatching purposes we use saline, trypsin, and Indirect Coombs techniques. We have for more than a year used the Indirect Coombs test on all compatibilities routinely as follows:

A cherry red saline suspension of donor cells is made and 2 drops of this is added to 2 drops of the patient's serum. It is centrifuged for 1 minute and read macroscopically. This is the saline crossmatch. If there is no gross incompatibility, a Coombs test is done on the same tube. It is incubated for 15 minutes at 37° C., then washed 3 times, draining dry after the third washing and one drop of Coombs serum is added. Centrifuge for 1 minute and read microscopically. We have found this technique quite simple and it has worked into our scheme of operation very satisfactorily. Of course, adequate centrifuging facilities are a prime necessity if this technique is to be adopted.

The main disadvantage of the Indirect Coombs test is that it takes a good deal of time. Before starting the method as it is now being used, we tested various incubation times, with the hope that it could be reduced. We found that the difference between 5 minutes incubation and 15 minutes was very marked. Very few antibodies could be detected within a 5 minute incubation period. However, the difference between a 15 minute incubation period and a 30 minute incubation period appeared negligible. We therefore decided that if we incubated our compatibilities 15 minutes, we would be detecting the majority of antibodies. There is no question that we may be missing some of the weaker ones, but if they are not picked up after 15 minutes of incubation, they are probably so weak that the danger to the patient is minimal.

We have used the standard cell, untrypsinated, in the Indirect Coombs technique with satisfactory results for those cases where blood is merely kept on reserve or for emergencies when there is not enough time for the full compatibility test. For example, if blood is requested for a patient, a G.I. bleeder or an A.P. bleeder where the exact time or need for the blood is unknown, the patient's serum is tested against this standard cell with the Indirect Coombs technique. If this proves negative, it is felt that blood can be given out after the saline compatibility alone has been done. This is the same procedure that is used in emergencies.

If an emergency develops where there is no time to complete the Indirect Coombs test on the compatibility or with the stand-

ard cell, the blood is given out without these tests being done and the tests are completed after the blood has gone out. The patient's serum, however, is always tested with the standard trypsinated cell since this takes only 1 minute and can detect most of the antibodies we may run across.

Through the use of the standard cell and the Indirect Coombs compatibility, we have detected many antibodies which would not have been picked up by any other compatibility technique. We have found Kell, Duffy, C, c, e, P, as well as many of the usual anti-D antibodies. We have had several cases of erythroblastosis in Rh positive mothers and we were able to anticipate these through the use of the trypsinated standard cell.

In routine testing of bloods to be kept on call, the following case was demonstrated.

Fig. 3

Case # 2

M.G. Female, Age 73.
Severe Anemia Hb—5 gm.
Pt.—Group O, Rh positive.
Donor—Group O, Rh positive.
Pt. serum X Donor cells (sal.) ....Compatible
Pt. serum X Standard cells (tryp.) ....Compatible
Pt. serum X Donor cells (I.C.) ....+ + +
Pt. serum X Cell Panel (I.C.) ....anti-Kell.

Today, with the increased use of blood it is almost essential that some modification of the more advanced techniques be used in all blood bank laboratories. These procedures are not difficult and they are not as time consuming as we first thought they would be. If one has adequate centrifuge facilities, there are no insuperable problems involved. In a small blood bank it should be quite a simple procedure to do an Indirect Coombs on every compatibility. In the larger blood banks if a choice has to be made, patients with histories of previous transfusions should definitely have this type of compatibility. The emergency situation, of course, is one that has to be handled separately. If a choice has to be made for the patient to bleed to death or the Indirect Coombs compatibility be done, I don't think anyone would hesitate which choice to make. We have used these techniques for more than 40,000 consecutive compatibilities, and we feel that they have not increased excessively the work of the blood bank, and we are enabled to furnish blood, which is compatible by the most advanced techniques available today. There will doubtless be many more blood antigens found and it remains to be seen whether even newer techniques will be required for their detection.

ENDOCRINE METHODS IN DIAGNOSIS*

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I should like to consider some of the tools which are available for the direct estimation of hormones or their excretory products. This will be a survey of the kinds of tests which can be done, rather than a more detailed consideration of a particular method.

Two general kinds of tests can be used in endocrinology. One is the biological assay, the other a chemical procedure. Bioassay technics depend on the response of animals to the substance being tested, using animals lacking in that particular hormone. Such tests require the careful use of control, untreated, animals and a large enough number of test animals so that errors due to variations in the responsiveness of the animal are not larger than the change which can be brought about by the hormone. When these requirements are met, the amount of hormone present can be measured quite accurately by using various dilutions of the test substance and comparing the response to that obtained from known amounts of the hormone, just as one does a chemical test. The chemical procedures used depend on the nature of the hormone in question.

The technics are chiefly colorimetric and depend upon the development of a characteristic color with the substance involved. Many steroids have characteristic absorption spectra which fact may be made use of in the colorimetric separation of fairly complex steroid mixtures. In other instances a more specific color reaction is utilized, such as the Zimmerman reaction for the measurement of 17-ketosteroids. Most of the absorption spectra are in the visible range, as the compounds formed may be blue, violet, or red. In some instances the ultraviolet portion of the spectrum is used or the flourescence of the compound may be measured. The positive identification of pure steroids may be accomplished by infra red spectrophotometry in the few laboratories which are fortunate enough to have this expensive and specialized equipment.

Chemical procedures may be refined by the prior separation of complex mixtures into their various components. This is done by taking advantage of the partition coefficient of the compounds and separating them by counter-current distribution. Similarly, paper or column chromatography may be used to obtain various fractions. The colorimetric procedures described or biological assays may then be applied to the individual fractions. There are some gravimetric procedures in use in endocrine chemistry when the steroid for which one is looking is separated out and weighed directly.

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It might be helpful to look at a diagram, which represents the relationships between the pituitary gland and the other endocrine glands, whose function it stimulates. The pituitary hormones are all proteins; four pituitary hormones are shown—growth hormone, which acts directly on growing tissues, thyrotrophic hormone, which stimulates the thyroid gland, ACTH, stimulating the adrenal cortex, and gonadotrophin, stimulating the ovary or testis. The target glands in turn secrete their hormones, shown in the arrows coming from each gland. These hormones are mostly steroids, and can be measured either chemically or by bioassay. In addition to affecting the tissues responsive to them, the hormones of the target glands act as a brake on the anterior pituitary, cutting down the amount of stimulating hormone produced. This is indicated by the interrupted arrows going back to the anterior pituitary. That the activity of the pituitary is influenced by the hypothalmus and the central nervous system is also shown.

Thyrotrophic hormone, for example, stimulates the thyroid to produce thyroglobulin, which in turn inhibits pituitary production of thyrotrophin (TSH). In evaluating a person's thyroid function then one would have to consider not only whether the gland was under or overactive, but also whether this was the result of something wrong with the thyroid itself or was the indirect result of too little or too much stimulation from the pituitary.

Tests for the amount of thyrotrophin in the urine can be done only in a few research laboratories at the present time, where one can test the response of the thyroid gland in animals whose pituitaries have been removed. The test is difficult because the small amount of protein hormone present in the urine must be separated from other substances, and is readily denatured by rough handling.

We are in a better position when we want to measure the function of the thyroid gland itself. There are of course many laboratory indications of thyroid function, such as the serum cholesterol and the basal metabolic rate. These are indirect reflections of thyroid activity. A more direct measure is the protein bound iodine or butanol extractable iodine of the blood. This depends on the fact that thyroglobulin is a protein which is rich in iodine. The protein bound iodine is measured colorimetrically as iodine. The technic is tedious and meticulous, for it measures very small amounts, 4 to 8 micrograms percent in the blood of normal persons, and can be in error unless all inorganic iodine is carefully separated. It is an accurate reflection of circulating thyroid hormone.

Another way to test thyroid function is by measuring the

capacity of the gland to take up iodine, which it uses in making thyroglobulin. This has become possible as radioisotopes have become available. A test dose of radioactive iodine, I_{131}, is given and after a suitable interval, usually 24 hours, the amount of radioactivity over the neck is measured with a Geiger counter. The normal person's thyroid "takes up" 20 to 60 percent of the dose given, and most of the rest is excreted in the urine. Therefore the radioactivity in the urine also reflects the activity of the thyroid; normally 40 to 80 percent of the test dose will be found in the urine within 48 hours. The hypothyroid person takes up less than 20 percent and excretes more than 80 percent, while a person with thyrotoxicosis will excrete only a small amount as the very active gland takes up more than 60 percent of the test dose. Although there is quite a bit of overlap between normal and abnormal, the test is of value especially in diagnosing hyperthyroidism.

It is possible, still as an experimental procedure, to distinguish between hypothyroidism due to pituitary failure and that due to disease of the thyroid gland. This is done by measuring the radioiodine uptake or the protein bound iodine and then giving the patient daily injections of TSH (which is made from the pituitaries of slaughter house animals). After a few days the test of thyroid activity is repeated. If the thyroid was normal it will have been stimulated to increase activity by the administered pituitary hormone.

The adrenal cortex depends on stimulation by pituitary corticotrophin, or ACTH. ACTH, a protein, can be measured, but only in a very few laboratories and only when the patient can spare 150cc of blood. The test is based on the effect of the blood extract on the adrenal glands of hypophysectomized rats—this is the ascorbic acid depletion test. When the adrenal is stimulated the amount of ascorbic acid it contains is sharply diminished.

The diagram indicates that the adrenal cortex produces two kinds of metabolic products, corticosteroids and 17-ketosteroids, which can be measured in the blood or urine, as an indication of the activity of the cortex. Blood tests, which are only now being developed, are preferable physiologically since they give a better indication of what is circulating in the body than does the amount which appears in the urine. Nevertheless there is a rather direct relationship between the activity of the adrenal and the hormones found in the urine. The tests used are either biological or chemical.

The bioassays have the advantage of providing information about actual physiological effect, but have the great practical disadvantage in that animals without pituitary glands must be used. Such animals are very difficult to care for and large num-

bers must be used for each test in the interest of accuracy. Such procedures therefore are expensive and time consuming and not suitable for most laboratories.

The chemical tests have their disadvantages too, as they depend chiefly on color reactions of purified extracts made from the urine or blood, compared with the color produced by the pure hormone for which one is testing. Extracts of biological fluids tend either to contain substances which contribute to the color developed although they are not hormones, or else to have lost some of the steroid content in the process of purification.

The corticosteroids and 17-ketosteroids have different biological effects, and can be considered separately. The corticosteroids are cortisone-like in their action so that they lower the eosinophile count, tend to raise the blood sugar and increase the excretion of uric acid. When the adrenal does not produce enough corticosteroid the eosinophile count may be high and there may be a tendency to hypoglycemia. This relationship is the basis of the Thorn test, as when ACTH is given the normal adrenal will secrete more cortisone-like hormone, and the eosinophile count will fall, while the uric acid excretion rises.

The measurement of corticosteroids in the urine is more direct than the eosinophile count.

There are two different chemical ways in which they can be assayed. Because of the structure of the compounds, the purified extract can be oxidized to formaldehyde which is readily measured colorimetrically, or it can be reduced by copper and measured in a modification of the Benedict method for sugar. Reduction can also be accomplished with phosphomolybdic acid and acetic acid. In either instance a 24 hour urine sample is needed. Normal adults excrete 0.3 to 0.7 mg of corticosteroids a day, while children excrete less in proportion to their body size.

Patients with Addison's disease (adrenal failure) or with failure of the anterior pituitary, so that the adrenal is lacking in stimulation, excrete very small amounts of corticosteroids. If the pituitary is at fault the urinary excretion can be increased by the injection of ACTH for a few days. In overactivity of the adrenal (Cushing's syndrome), due to either a tumor of the adrenal or exces stimulation by the person's own ACTH, much larger amounts of corticosteroids will be found in the urine.

The 17-ketosteroids coming from the adrenal reflect adrenal "androgen" production; these compounds are responsible for the growth of pubic and axillary hair and for acne. In excessive amounts they may cause virilism, so that a woman or child takes on some of the physical characteristics of an adult man. Determination of the 17-ketosteroid excretion requires a complete 24 hour urine collection and is based on color development in a

purified extract of the urine. Normal women excrete about 8 to 15 mg a day, and men 12 to 20 mg a day. Larger amounts may be found when the patient has an adrenal tumor or hyperplasia of both adrenals due to excess ACTH stimulation.

In pituitary or adrenal insufficiency 17-ketosteroid excretion is low, just as is corticosteroid excretion. It can be increased by ACTH only if the primary fault is in the pituitary rather than the adrenal. When the adrenal is underactive both corticosteroid and 17-ketosteroid excretion is usually reduced. In overactivity of the adrenal both kinds of steroids may be increased in the urine, or one of them may predominate.

Pituitary gonad stimulating hormones—gonadotrophins—can be considered as a unit. Actually there are probably three kinds. One is follicle stimulating hormone (FSH) which causes the ovary to produce ova and estrogens and the testis to produce sperm. Luteinizing hormone (LH) stimulates ovulation in the female and the production of male sex hormone by the testis. Luteotrophic hormone (LTH) stimulates the ovary to produce progesterone. Most laboratory tests for gonadotrophins do not distinguish between the three kinds but measure the mixture in the urine. This is one of the oldest and best established tests for a pituitary hormone. The protein is separated from the urine and is assayed, after some purification, by the effect of the extract on the ovary of the immature mouse. The results are therefore expressed as mouse units per 24 hours.

The normal adult man or woman excretes 13 to 56 mouse units per day. This test can be used in diagnosing the cause of failure of normal sex development. If the gonadotrophin content of a urine sample is low it means the pituitary is not producing much of this hormone. If the assay is abnormally high, as 92 or more mouse units a day, it means that the pituitary is functioning but that the gonad is not responding. This is normal in old age. As with the relationship of the other target glands to the anterior pituitary, the hormones produced by the ovary or testis inhibit the pituitary secretion of gonadotrophin. If the gonad is not functioning, this check is lacking and excess gonadotrophin appears in the urine.

The testis secretes androgens, which cause male sex development and are excreted in the urine as 17-ketosteroids. They can be measured by bioassay or chemically, as mentioned before. The reason men excrete more 17-ketosteroid than women do is just because some of the hormone comes from the testis in the male, and from the adrenals in both sexes.

The ovary secretes two kinds of hormones, estrogens and progesterone. Estrogens cause most of the femine sex development. They are still best measured in the urine by biological assay, as the older chemical methods were not very sensitive. Rela-

tively recent advantage has been taken of the fact that the estrogens are naturally fluorescent, and they can be determined by ultra-violet absorption after they have been separated out from other fluorogenic compounds by rather complex organic separations, which can be carried out by counter-current distribution. Clinical means often suffice as indications of estrogen activity. Progesterone is the ovarian hormone which is essential for normal menstrual cycles and for successful pregnancies. It is excreted in the urine as pregnanediol which can be measured colorimetrically or by weighing the precipitated steroid.

Growth hormone, which is shown on the extreme right of the slide, is different from the other pituitary hormones in that it acts directly on the growing tissues rather than through another gland. It will be of great interest to measure growth hormone when sensitive technics become available. So far the assay methods can be used only to measure the amount of growth hormone in a pituitary extract, but not in any biological fluid. It is used for standardizing growth hormone preparations. This measure is carried out in young rats whose pituitaries have been removed. Such animals stop growing, and have a narrow zone of new cartilage (the epiphyseal plate) at the ends of the long bones. The response to growth hormone is gauged from the increase in width of the epiphyseal plate as growth is resumed.

This survey has included only the biological and chemical methods available for the direct estimation of hormones or their excretory products, with some effort to indicate the normal phyiological relationships. Insulin has been deliberately left out, as it will no doubt be discussed by other people. The parathyroids have simply been left out, as have all the clinical and indirect chemical methods of evaluating endocrine function.

PRINCIPLES AND TECHNIQUES OF THE SPECTROPHOTOMETER AND THE FLAME PHOTOMETER*

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Spectrophotometry is rapidly becoming for the medical technologist one of the most versatile and popular methods of analysis. Among the techniques now being used in clinical analysis as a result of the development of the spectrophotometer are: absorption spectrophotometry (colorimetry, ultraviolet and infrared absorption measurements); emission spectrophotometry (flame photometry) and fluorescent spectophotometry. All of these analytical techniques have been developed from the fundamental theory of the response of molecules to electromagnetic radiations.

The usefulness of each analytical method is limited by the nature of the response of the molecules being studied to a specific portion of the electromagnetic spectrum. The spectrophotometer is a precision instrument designed to measure accurately the response of the molecules under study to energizing radiations.

Though there are several satisfactory spectrophotometers now on the market, the remarks in this paper will be confined essentially to the two models in use in the author's laboratory, the Coleman Junior Spectrophotometer, Model 6-A and the Beckman Quartz Spectrophotometer, Model DU. The latter is one of the most versatile spectrophotometers available. Its range of usefulness includes absorption, fluorescent and flame photometry. The medical technician utilizing the spectrophotometer in making any of these measurements will be working principally in that portion of the electromagnetic spectrum from 2000 to 8000 Angstrom units—a comparatively small working area, Table 1. Spectral measurements in the infrared, far ultraviolet and x-ray regions have not as yet found practical use in the clinical laboratory.

Absorption spectroscopy in the visible region of the spectrum, i.e., colorimetry, depends essentially upon that property of a colored compound to absorb a given amount of radiant energy of a specific wave length to which it has been exposed. The color of the unknown may be characteristic of the compound itself as in hemoglobin, or it may be developed in the presence of a second compound which is itself colored, e.g., diphenylthiocarbazone, or is not colored, e.g., thiocyanate salt.

It is of interest to note that the improvement of instruments for measuring these color complexes has far exceeded the advance being made in the development of new chemical reactions and the understanding of those now being used to produce these complexes. At present, then, the application of the spectrophotometer in the field of colorimetry is limited not by lack of proper instrumentation but by lack of knowledge of needed color reactions.

To be suitable for spectrophotometric analysis the colored

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system must meet certain requirements. Important among these are: 1) Rapid development of color is not only convenient but avoids effects of temperature and other factors on the reaction. A fairly intense color is necessary in detecting small amounts of an unknown substance. In higher concentrations the color can always be diluted and appropriate calculations carried out. 2) The color reagent itself should be stable and the color developed should likewise be stable sufficiently long to permit accurate measurement. 3) Freedom from influence by variations in temperature, pH and other environmental factors is desirable. Reagents or systems sensitive to such influences require careful control and/or application of correction factors. 4) Solubility of the reagent and reaction products is essential for exact measurements. Colloidal or turbid solutions give inaccurate readings due to non-specific absorption and light scattering and susceptibility to other constitutents. 5) The maximum absorption of the colored complex must be definite and within the range of the spectrophotometer. 6) The color developed must be specific for the compound or element being analyzed. All interfering ions must be eliminated. Non-specific reactions introduce considerable error. 7) Conformity of the colored system to Beer's Law though not absolutely essential is extremely useful and simplifies calculation of results.

A thorough understanding of the general laws of absorption is important in any discussion of the principles upon which the spectrophotometer is operated.

When a compound or element absorbs light in the visible or near ultraviolet region of the spectrum outer electrons in the atomic orbits are displaced. The type and number of displacements and amount of light absorbed are characteristic of the individual molecules. The only difference between the absorption of ultraviolet or visible light by the molecules lies in the fact that greater energies and larger displacements are characteristic of ultraviolet absorption. In the visible region 35,000 to 71,000 calories per mole are required for absorption. In the ultraviolet this value rises to several hundred thousand (1).

Lambert and Beer have formulated two fundamental laws which express the relationships involved in this absorption phenomenon. Lambert's[1] Law states that a definite relationship exists between the absorption capacity of a material and the *thickness* of the absorbing medium. The extent of the absorption also depends upon the probability that the energy of the quantum will be transferred to the molecule effecting the displacement. Lambert has expressed the probability by a mathematical constant in the equation:

$$\frac{dI}{I} = -k' \, db \qquad (1)$$

In this equation, I is the intensity of the light in terms of the number of quanta per second, per square centimeter hitting the surface; dI is a differential expressing the change in intensity of the light after passing through a thin layer of the absorbing medium, db. The value of k′ varies with the wave length of radiant energy applied to a given depth of absorbing medium. At a given wave length the intensity of the transmitted light, I, after having passed through "b" centimeters of absorbing medium can be related to the initial intensity, I_o, by integration of the equation between the limits: I_0 when $b = O$; and I at thickness b.

$$\ln I/I_o = -k'b \qquad \text{(II)}$$

A second law, fomulated by Beer, states that the intensity of the transmitted light is also a function of the *concentration* of the absorbing material. If the length, b, of the absorbing material is held fixed, then according to Beer's Law:

$$\frac{dI}{I} = -k'' \, dc \qquad \text{(III)}$$

c represents concentration of the absorbing material.

The two laws are usually expressed in a single equation. By combination of the integrated forms of Equations I and III:

$$\ln I/I_o = -k\,bc \qquad \text{(IV)}$$

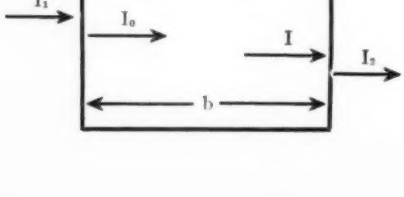
In this equation, k becomes the absorption coefficient of the sample. Most spectroscopists prefer to work with the equation expressed in the logarithim to the base 10. Conversion of Equation IV from the natural logarithim gives:

$$\log_{10} I/I_o = -a_s bc \qquad \text{(V)}$$

in which a_s is now the absorbancy index. When $\log_{10} I/I_o$ is plotted against concentration, c, a straight line is obtained, Figure 4.

Symbols and terminology in absorption spectroscopy have become considerably confused as the result of varying usages adopted by many research workers. The national Bureau of Standards has issued certain recommendations which are being widely accepted and which help to clarify the interpretation of published spectrophotometric data and instructions for use of spectrophotometers though as yet not all such instructions or publications conform completely to the N.B.S. terminology. The symbols and their definitions as suggested by the Bureau of Standards (4) are as follows:

Fig. 1. Schematic Diagram of the Transmission of Radiant Energy



In the following derivations only *ratios* of radiant energy are considered.

- I_1 — radiant energy incident upon the first surface of the sample
- I_0 — radiant energy entering the sample
- I — radiant energy incident upon the second surface of sample
- I_2 — radiant energy leaving the sample
- T — I_2/I_1, transmittance of the sample
- T_i — I/I_0, internal transmittance of the sample
- A_i — $-\log_{10} T_i$, $\log_{10} 1/T_i$, $\log_{10} I_0/I$, absorbance of sample
- a_i — A_i/b, absorbance index of sample ($b =$ length of absorbing path)

The above terms refer to the rectilinear transmission of homogeneous radiant energy (monochromatic light) through a homogeneous, isotropic, non-metallic medium having plane, smooth, parallel surfaces. However, the terms most useful to the clinical analyst are those relating to a non-metallic medium such as a liquid or solution confined between the windows of a cell. This is the relation measured by the spectrophotometer.

- $T_{soln.}$ — (over-all) transmittance of a given cell containing the non-metallic medium of which the component of interest is the solute or one of the constituents.
- $T_{solv.}$ — (over-all) transmittance of the same or duplicate cell containing the pure solvent or all of the constituents in the same proportions minus the one of interest.
- T_s — $T_{soln.}/T_{solv.}$, transmittancy of the sample (T_s does not exactly equal $T_{i\ soln.}/T_{i\ solv.}$ but for all practical purposes this difference is negligible and $I/I_0 = T_s$).
- A_s — $-\log_{10} T_s$, absorbancy of the sample (replaces older terms: optical density, D; or extinction, E).
- a_s — A_s/bc, absorbancy index (replaces the extinction coefficient, k).
- a_M — molar absorbancy index (replaces the molar extinction coefficient, K or ε).

Figures 3 and 4 illustrate the relationships between the terms just discussed and older terminology still in use. The curve in Figure 3 was run as a preliminary step in setting up a calibration curve for the determination of mercury. The mercury reagent used was s-diphenylcarbazide (5). Concentration of the mercuric ion in the mercuric acetate standard, 4.98×10^{-5}M. A Coleman Universal Spectrophotometer, Model 11, was used for the experiments graphed in Figures 3 and 4. Transmission and optical density values were read directly from the spectrophotometer scale. The molar absorbancy indicies were calculated from the Lambert-Beer Equation (V). Readings in Figure 4 were made at the absorption maximum of 530 mμ. as determined from the data of Figure 3. As seen from Curve 3, Figure 4, when linear values such as I/I_0 are used a straight line is not obtained.

The combined optical and electrical systems of the spectrophotometer are designed so as to provide the incident radiant energy necessary and to measure that fraction of incident energy absorbed by the colored solution in terms of the light transmitted by the sample.

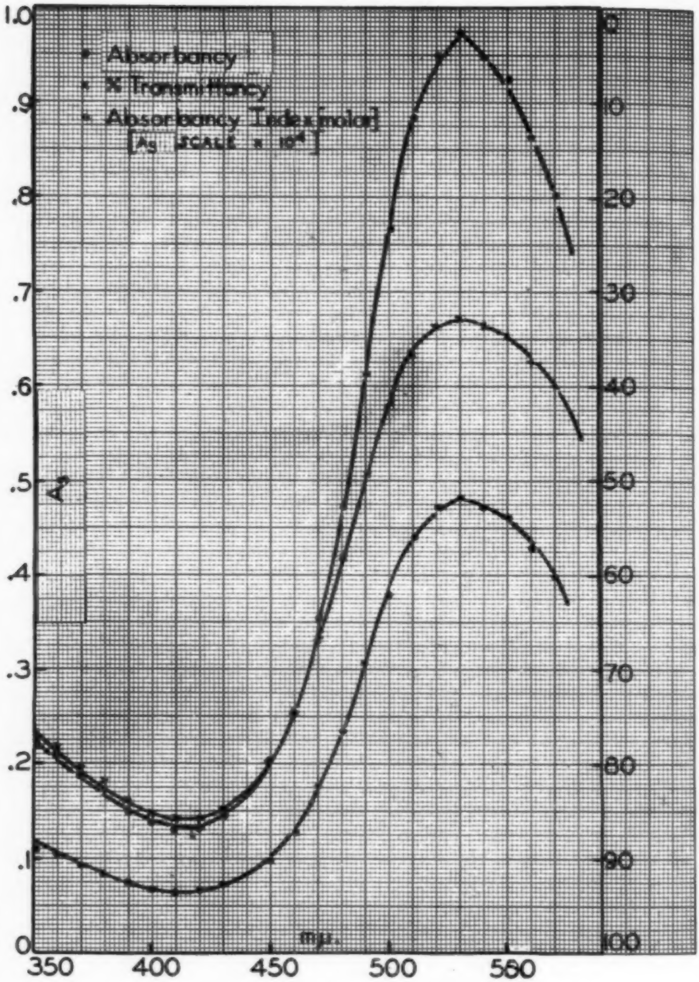


Fig. 3. Spectrum of mercuric ion — s-diphenylcarbazide complex.

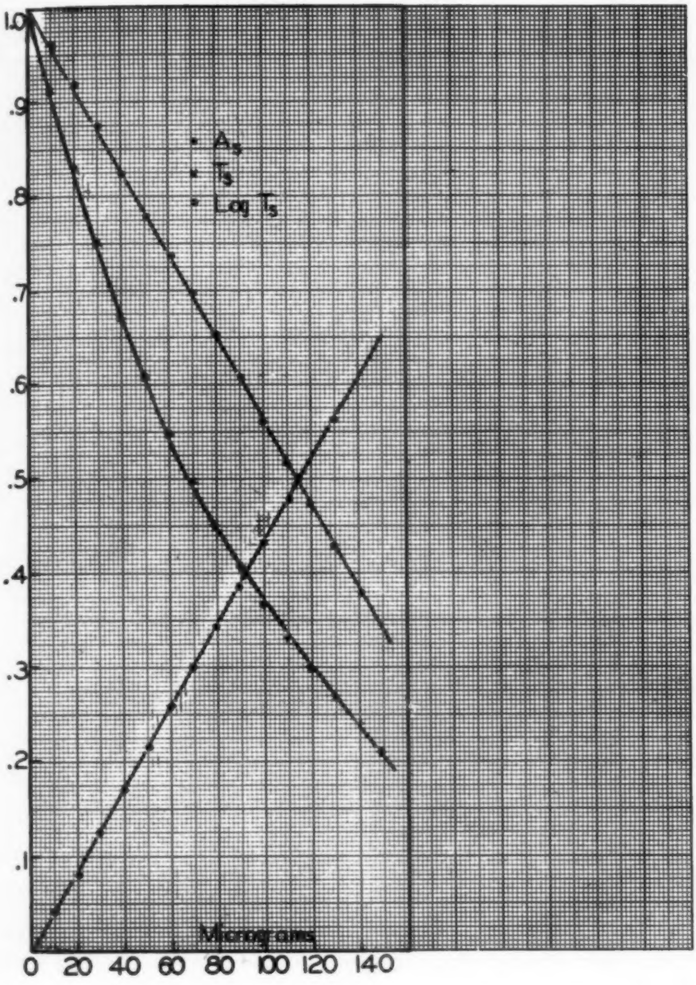


Fig. 4. Mercury calibration curve. ○ Curve 1, ordinate, log I/I_o; • Curve 2, ordinate, log I_o/I; X Curve 3, ordinate, $T_s(I/I_o)$

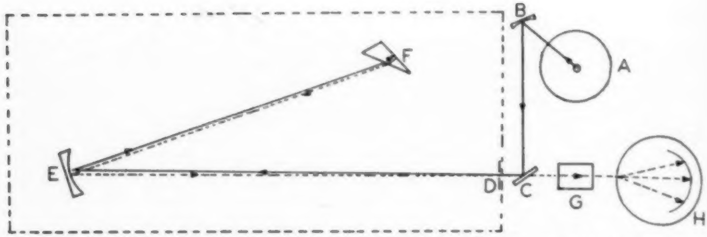


Fig. 5. Optical system of the Beckman quartz spectrophotometer Model DU. Reproduced by permission of *J. Opt. Soc. Amer.* (6).

The two basic essentials of a good spectrophotometer are: a proper light source and an adequate monochrometer. Figure 5 shows the optical system of the Beckman Model DU Spectrophotometer (6). This system is presented as typical of the modern spectrophotometer. The light source is located at A. A tungsten lamp provides radiation for measurements in the visible region and a hot-cathode hydrogen discharge tube for measurements from 350 mμ. to 220 mμ. An image of the light source is focused by condensing mirrors at B and C through the entrance slit at D. This slit is located directly below the exit slit. The slit width of the Beckman instrument is adjustable. This feature is a major advantage of this model in certain types of work (7). Slit width seriously affects spectral resolution. In Figure 6, Curve 1 was run on a Coleman Model 6-A Spectrophotometer having a fixed slit width giving a nominal band width of 35 mμ. The wave length scale of this instrument is calibrated only to 5 mμ. divisions. Consequently, the sharp band at 582 mμ. is not detected with this instrument. Curve 2 made with a Beckman Model DU Spectrophotometer at a very narrow adjustable slit width which gives a nominal band width of less than 1 mμ. shows definite resolution of the maximum at 582 mμ. The wave length scale of this instrument is calibrated to 0.5 mμ. in the ultraviolet region and to 2.0 mμ. in the visible region.

The second requirement of a good spectrophotometer is the spectral purity of the light source. This means a well designed monochromator. In the Beckman instrument a quartz prism is used. In the Coleman instrument a replica grating serves as monochromator. Though the grating is less expensive and provides higher dispersion in the visible and infra red regions, it has a decided disadvantage in the high amount of scattered light it produces. Since stray light is particularly undesirable in spectral work, the quartz prism is preferred for precision work. The replica grating is satisfactory for accurate clinical work.

The light beam entering slit D is then collimated by mirror E and passes through the quartz prism to the reflecting surface F.

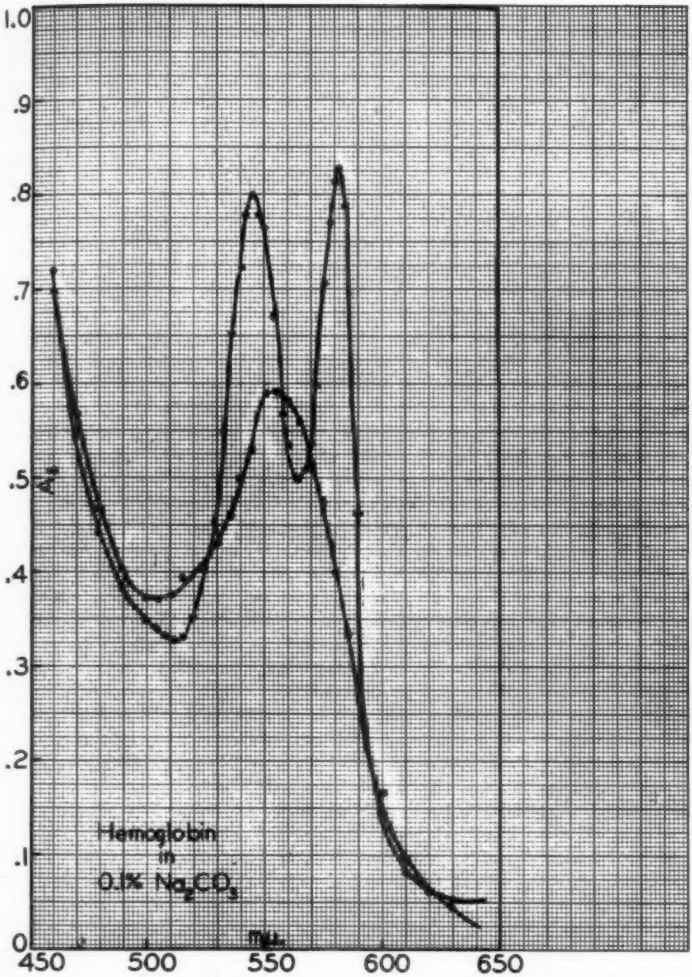


Fig. 6. Hemoglobin curves. Concentration, 41 gm.%; • Curve 1; ○ Curve 2

After reflection at F the monochromatic beam returns along nearly the same path. It emerges directly above the entrance beam and passes through the cell at G which contains the ab-

sorbing material. The light transmitted by the solution strikes a sensitive phototube at H. The use of two interchangeable phototubes increases the sensitivity of the Beckman instrument. A cesium oxide, "red sensitive," tube is used in making measurements above 625 mμ. A special cesium antimony, "blue sensitive," tube is used for measurements below 625 mμ. The electrical response of the tube is proportional to the quanta of light energy incident upon it. This current is then amplified and measured directly by balancing the voltage drop developed in the 2000 megohm resistor. A slide wire potentiometer is used for this purpose and the balance is indicated on a milliammeter. The potentiometer has a double scale calibrated in terms of optical density and transmission which are read directly.

Accurate measurements also depend upon the selection of a proper reference. Ordinarily, a reference should consist of all the constituents of the sample to be measured with the exception of the constituent under consideration. Improper selection of a reference can introduce considerable error in the measurements. A simple test for corrective efficiency of the blank is to observe whether the curve passes through the ordinate at zero optical density when the concentration of the constituent being measured is zero. Automatic correction for non-selective absorption of solvent and reagent by the modern spectrophotometer is one of its many conveniences. The correction consists in adjusting the slide wire potentiometer scale to read zero density when the blank containing the reference solution is in the light path.

Another field of spectral analysis gaining in popularity in the clinical laboratory is flame photometry. This technique makes use of that property of molecules which enables them to *emit* energy previously absorbed. Measurement of the emitted energy both qualitatively and quantitatively constitutes emission spectrophotometry. It has been pointed out that in absorption spectrophotometry certain electron displacements occur when molecules are exposed to specific wave lengths of electromagnetic radiations. These electrons which are displaced to higher energy levels tend to return to lower energy levels emitting the excess energy absorbed in their movement to the higher energy levels. This energy may or may not be of the same wave length as the absorbed energy. The energy emitted is usually of several wave lengths with one or two bands predominating. Sodium molecules, for example, on being energized with infrared (heat) radiations in a flame emit radiations at the 588.9 and 589.5 mμ. bands. Potassium emits principally at 404.4, 404.7, 766.4 and 769.9 mμ. The wave lengths of 589 mμ. and 768 mμ. are used for determination of these two elements in the flame photometer. The intensity of the energy emitted at a particular wave length is a measure of the concentration of the element present.

The Beckman Model DU Spectrophotometer can also be utilized as a flame photometer. Essentially the same optical system is employed. The chief modification consists in the use of an atomizer-burner supplied with an acetylene-oxygen or hydrogen-oxygen fuel instead of the tungsten lamp or hydrogen discharge tube as energy source. For a detailed discussion of the technique and principles of the flame photometer, the reader is referred to Seger, Van Loon and Merle (8)

In those laboratories where a large number of sodium and potassium determinations are made use of the flame photometer saves both time and expense. The instruments now available measure sodium and potassium in biological fluids sufficiently accurate for hospital use. However, in the opinion of many workers (9) titration and gravimetric procedures will remain the standards against which determinations with the flame photometer are checked.

As yet the determination of calcium and magnesium cannot be carried out as satisfactorily as sodium and potassium. In fact, Archibald (9) feels that is is unlikely that determination of the alkali earth metals by flame photometry will prove as popular or as practical as the determination of sodium or potassium ions. The use of the flame photometer in the clinical laboratory is then, for the present, quite limited. Glick (10) believes that flame photometry will be displaced by emission spectroscopy in which a variety of elements can be identified simultaneously and quantitatively in a very small single sample. He forecasts that this analytical method will be brought to practicality for the laboratory technician in the near future.

Another application of the spectrophotometer is its use in the determination of prothrombin time as reported by Confortini and Degradi (11).

The possibilities of adaptation of absorption colorimetry to micro-chemical analyses have received considerable attention by research workers during the past few years (9). Glick (10) has designed a microscope colorimeter employing capillary cuvettes which are sealed to a microscope slide and covered with a cover glass. Use of such capillaries permits measurements on as little as five microliters of solution.

The microscope spectrophotometer-fluorimeter unit shown in Figure 7 also designed by Glick is an example of the type of equipment which will possibly be included in the future armamentarium of the medical technologist.

Development of such specialized spectrophotometric equipment serves as a striking summary of the progress made in absorption and emission analyses from the days when the Dubosque Colorimeter was the answer to the perennial plea of the medical technologist for improved methods in clinical chemistry.

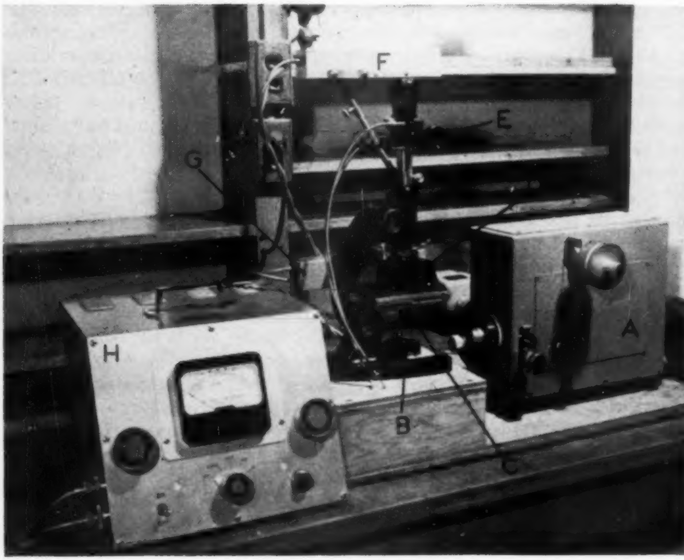


Fig. 7. Microscope Spectrophotometer-fluorimeter Unit. Reproduced by permission of Dr. Glick and the editor of *Chem. and Eng. News*. A. Grating monochromator with quartz condensing lenses; B. aluminum first surface reflector; C. collimating tube with aligned pin holes at each end; D. light-tight housing containing mechanical stage with external control knobs for centering capillary cuvettes with quartz cover slips on quartz microscope slide; E. observation eyepiece to aid in positioning of cuvette to place lumen in optical axis; F. replaceable photoelectric unit for absorption measurements sensitive in visible or ultraviolet region depending on requirement; G. photoelectric unit for fluorescence measurements sensitive in visible region; H. amplifier-galvanometer unit for measuring photocurrent.

TABLE I
ELECTROMAGNETIC SPECTRUM

Wave Length Å	<.0001	.01—1.0	1.0—100	100—4000	4000—8000	8000—10^7	10^7—10^{13}	>10^{13}
Type of Radiation	Cosmic rays	Gamma rays	X-rays	Ultraviolet rays	Visible Light	Infrared rays	Radio waves	Alternating currents

1 Ångstrom unit = 0.1 millimicron.
Working range of Coleman Model 6-A Junior Spectrophotomer, 400—700 mμ.
Working range of Beckman Model DU Quartz Spectrophotometer, 200—2000 mμ.

[1] According to Mellon (2) though commonly attributed to Lambert, this law was first formulated by Bouguer (3).

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MORE CLASSIFIED ADS

LABORATORY TECHNIQUES FOR THE DIAGNOSIS OF VIRAL DISEASES*

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Painstaking and devoted research by many investigators in the field of virus diseases since the discovery of tobacco mosaic virus in 1892, has lead to a greatly increasing understanding of the etiology and epidemiology of the more common human virus infections. Application of fundamental discoveries to the development of laboratory techniques which may aid the physician in diagnosing viral maladies is being realized more each year. Since the last war, and perhaps as a result of the stimulus of Armed Service laboratories, virus laboratories which can be utilized by the physician and epidemiologist have been established in several states. The Virus and Rickettsia Section of the Public Health Service, Communicable Disease Center, is available as a reference laboratory to these state virus laboratories. It also accepts specimens for diagnostic tests which are submitted through State Laboratories by local medical and health department personnel. The Ohio Department of Health has established a state virus laboratory within the past year, and I am speaking to you as the administrator of that laboratory, but not as an authority in the field of virology. I appreciate the opportunity that you have given me to point out how you may best utilize the services of a virus laboratory and to outline some of the available techniques.

It should be emphasized that, at the present time, results of virus tests cannot often be of use to a physician in treating or diagnosing a particular patient. Serological procedures indicate significant antibody only during convalescence. Virus isolation is often time consuming. Hence, diagnosis in retrospect is the usual rule, but the value of this should not be underrated. Other patients with similar symptoms may be benefitted, and the practice of medicine thus improved. For example, by means of a complement fixation test, Sigel[1] discovered an outbreak of Q fever which had been thought to be "flu" in a wool processing plant, and Irons *et al.*[2] similarly revealed an outbreak of ornithosis in workers who handled turkeys in a poultry plant. Thus, the epidemiologist may know what virus diseases are prevalent and in what localities, and be able to institute effective control and preventive measures, helping the community even more than the patient. An example, which we will consider later is the international and national influenza study program. The value of

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* Read before ASMT, Louisville, Ky., June, 1953.

viral identifications in biological warfare must also be kept in mind. The possibility of discovering "new" virus diseases is always present. In addition to routine testing, the alert virus laboratory should also give attention to studies for the improvement of current techniques.

The establishment of a virus laboratory must be looked upon as a rather costly project, not only for original equipment, but also for maintenance. One cannot expect a high yield of positive findings. For this reason, only the well endowed institutions can support a complete virus facility. However, any laboratory which performs complement fixation tests for syphilis can, with little difficulty, use those virus and rickettsial antigens which are available and thus include the serological part of virus laboratory services. This can be done in larger hospitals or private laboratories. These antigens are relatively expensive, and supplies of positive control sera are limited and difficult to obtain. The latter problems will, no doubt, be resolved by our biological manufacturers, when there is sufficient demand for these products.

The laboratory which attempts virus isolation procedures requires additional equipment and personnel. Animal facilities for rabbits, guinea pigs, and mice, and perhaps for monkeys should be available, and a constant supply of healthy stock must be maintained. Mice are most commonly used. Equipment items that are necessary include an egg incubator, egg candler, electric drill, and probably a high speed refrigerated centrifuge, tissue blendor, and large supplies of syringes, needles, and autopsy instruments. An enclosed working chamber or hood where infective materials may be safely handled is also advisable. This should be equipped with an exhaust system which sterilizes the air, and an ultra-violet lamp for sterilization of the interior of the working chamber. A super-cold freezer cooled either electrically or by dry-ice is essential for keeping stock virus strains.

Let us now turn to specific techniques that are available to the virologist in the diagnostic laboratory. First we shall consider the immunological reactions. Koprowski's[3] review of these tests serves as a classic reference. They may be divided into complement fixation, hemagglutination, and neutralization procedures. For all serologic procedures it is essential to have two specimens of the patient's clotted blood, one drawn during the acute phase of illness, and a second or series of specimens drawn during convalescence, not sooner than ten days to two weeks after the first. Acute phase sera may best be stored in the frozen state. It is often difficult to obtain the convalescent blood specimen, for at this time the patient may be well and not available to the hospital or physician, but this sample is the important one, and the hospital should certainly obtain a specimen prior to releasing the patient. Only a rise in antibody titer is positive

evidence of recent infection, as frequently there is an initial level of antibody normally present in the acute specimen as a result of immunization or of previous infection. These paired samples are best tested in the same protocol, rather than separately as collected in order to obviate day to day fluctuations in test sensitivity levels. The physician must be made to understand the need for the convalescent specimen.

Specific viral and rickettsial complement fixing antigens may be purchased commercially. Some of the better equipped laboratories prepare their own, but this is often a difficult or dangerous procedure. Viral encephalitis antigens may be prepared from infected mouse brains or whole chick embryos. Extraction with benzene or acetone-ether renders the antigens specific and non-anticomplementary. Mumps antigen of chick embryo allantoic fluid contains viral antigen, but with addition of chorioallantoic membranes, "soluble" antigen is obtained, and in mumps infection a rise in titer to soluble antigen occurs early in the disease.[4] Lymphocytic choriomeningitis antigen consists of infected guinea pig lung suspension. Psittacosis or lymphogranuloma venereum antigen may be prepared from infected yolk sacs or phenolized chorioallantoic fluids and membranes. Cross reactions occur between this psittacosis-lymphogranuloma group of viruses. Rickettsial antigens are generally made from chick embryo yolk sacs by ether extraction, benzene treatment, and precipitation with sodium sulfate, according to the method of Van der Scheer, Bohnel, and Cox.[5] Unless non-specific factors are removed by this process, the antigen often gives reaction with Wassermann-positive human syphilitic serum.

The complement fixation procedure for these tests is generally based upon 100 per cent endpoint titrations by a modified Kolmer technique, although the 50 per cent endpoint methods are readily applicable and are advantageous from the standpoint of reproducibility. One should strictly adhere to the technique recommended by the manufacturer of the antigen, and use antigen at the titer recommended, unless a separate antigenic titration is performed for a modified test. This is usually not practical when one considers the cost of commercial antigens, and the scarcity of hyperimmune antisera. The block titration of antigen should be carried out in all cases, if the technique is modified or if antigen is prepared locally. A control of normal antigen from non-infected tissue should be included in the protocol, but when such material is unavailable one may employ a second viral antigen which is prepared from the same tissue source. Other controls in the test are serum controls (positive, negative, and patient's), viral antigen control, complement control, and sheep cell control.

Complement fixation results should be interpreted in light of

the clinical history of the patient, the incidence of inapparent or abortive infections, vaccination history of the patient, and cross reactions with other virus agents. A four-fold or greater rise in titer of the convalescent serum sample over the acute phase serum titer is generally considered to be of significance.

Recently experimental complement fixation tests for poliomyelitis have been reported by Svedmyr, Enders, and Holloway.[6] They prepared antigens from tissue cultures in which the three prototype strains of poliomyelitis virus had been propagated. The antibodies were found to be type specific for monkeys, but not for human cases, and the concentration of antibody decreased markedly 1.5 to 3 years after illness.

Certain viruses possess the property of agglutinating erythrocytes, notably influenza, mumps, Newcastle, variola, vaccinia, and some encephalitis viruses. This hemagglutination phenomenon is utilized primarily in the diagnosis of influenza infection. Fowl erythrocytes are readily agglutinated by influenza viruses, and a quantitative measurement may be made by photometric methods, Hirst and Pickels,[8] or by pattern endpoints of agglutinated cells, Salk.[9] The hemagglutination-inhibition test, or the inhibition of viral agglutination by specific antibody in patient's serum is used on paired serum specimens to determine rise in titer of influenza antibody. A constant amount of virus is mixed with two-fold dilutions of serum. Chicken erythrocyte suspension is added, and the inhibition of agglutination is read after 60 minutes. The technique employed in our laboratory is that of the Committee on Standard Serological Procedures in Influenza Studies.[10] Lytle and Woolridge[11] prepared purified and stable virus suspensions for routine use in serologic tests for influenza by methanol precipitation of infected allantoic fluids, and subsequent elution in 0.1 M mono and disodium phosphate buffer, pH 7.0.

Controversy exists as to the preference of complement fixation over hemagglutination tests in the laboratory diagnosis of influenza. It has been shown[12] that the complement fixation test using virus strains of broad antigenic composition as antigens would provide the ideal diagnostic test for the presence of influenza infection. However, the hemagglutination-inhibition method is more likely to demonstrate which specific strain is responsible for the infection.

Of practical consideration is the fact that influenza complement fixation antigens are usually of low titer, and hence large quantities of antigen are used in this procedure, in contrast to that needed in the hemagglutination-inhibition test. The fact that the influenza infections from year to year are caused by antigenically different strains complicates the picture, and requires attention to the strain specificity of antigens in the hemagglutination-inhibition test.

One cannot discuss influenza without mentioning the influenza study program in the United States, which is a part of that of the World Health Organization.[13] Collaborating area laboratories such as ours serve as lookout posts, and promptly report the serologic findings and virus types indicated. The influenza center receives and disseminates this information. The strain study center for the Americas determines antigenic variation in strains of influenza virus that are isolated.

The virus neutralization test consists most frequently of the admixture of serial dilutions of a living virulent virus with constant amounts of patient's serum, and after a suitable incubation period, inoculation of the mixture into a susceptible animal host. A similar series of dilutions is prepared using known negative serum. The test requires large numbers of animals, usually mice, but fertile hen's eggs have been used. The results are often expressed as the neutralization index, or the ratio of the highest concentration of virus against which the patient's serum will protect, to the highest concentration of the virus to which the normal serum gives protection. In addition to testing for viral antibodies in patient's sera, neutralization tests may be used for the identification of viruses, and for studies of antigenic relationships.

The neutralization test is of particular value for viral encephalitis infections. Storage of test sera, time and temperature of incubation, and the age of test animals have been found to have bearing on the outcome of these tests. The neutralization test has been applied also to identification of Coxsackie, poliomyelitis, psittacosis, herpes and dengue infections or viruses. Recently a test for poliomyelitis neutralizing antibody has been performed by the tissue culture method of Robbins, Enders, *et al.*[7]

A second group of techniques of the virus laboratory are those in which viruses are actually isolated and identified. Virus isolation procedures should be reserved for epidemics, or cases of special interest, and for fatal infections. Materials for isolation of virus must be freshly processed, and where possible collected under sterile conditions. Any of the following may be sources of virus: Throat or nasal washings, sputum, blood serum, effusion fluid, tissue, or lesion scrapings. Their selection is determined by clinical indications. No preservative should be added to any material. Spinal fluid is usually a poor source of virus, and should not be obtained. Sometimes arthropods, or birds wrapped in gauze soaked in formaldehyde or phenol are selected for this study. All such samples must be quickly frozen, preferably in sealed glass ampoules, or in tightly stoppered containers sealed with paraffin. They are then packed in dry ice and shipped frozen to the virus laboratory without delay. Tissue may alternatively be preserved in buffered glycerol saline solution. All virus speci-

mens should be accompanied by data giving the disease suspected, date of onset, and a brief clinical history of patient and other similar cases in the vicinity.

Viruses are obligate, intracellular parasites, and hence, cannot be cultivated in the absence of living cells. Direct microscopic examination for viral inclusion bodies is practiced for rabies infection, but this procedure must often be supplemented by intracerebral mouse inoculation of the microscopically negative brain material.

For virus isolation, the appropriate material is first put in the fluid state by a grinding or homogenization process in a saline diluent containing serum or bovine albumin. Penicillin and streptomycin are generally added to inhibit possible bacterial contaminants.

The suitable living tissue for virus multiplication is then selected. This, very frequently, is the developing chick embryo,[14] and the route of inoculation may be into the allantoic sac, amniotic sac, chorio-allantoic membrane, or yolk sac. The presence of a virus may be indicated by death of the embryo, appearance of characteristic lesions, demonstration of inclusion bodies, or by means of the aforementioned complement fixation, hemagglutination, or neutralization procedures, or by specific protection tests.

Laboratory animals such as the white mouse or rabbit are also frequently used. Specific techniques and routes of inoculation are given in the American Public Health Association Diagnostic Procedures for Virus and Rickettsial Diseases.[15]

TABLE I
Selected Serological Results

Antigen	Titer Acute Phase	Titer Convalescent Phase	Remarks
Complement Fixation Tests			
Lymphocytic choriomeningitis	Negative	Negative	Exposure to mumps 5 weeks prior. 6 days of fever. Doubtful neurologic findings.
Mumps	1:32	1:128	
Lymphocytic choriomeningitis	Negative	Negative	Mumps meningo-encephalitis.
Mumps	1:4	1:64	
Rocky Mountain spotted fever	1:32 (After 21 Days)	1:128 (After 31 Days)	Guinea pig inoculated with tick suspension.
Rocky Mountain spotted fever	Negative	1:16	Clinical Rocky Mountain spotted fever.
	Sample	**Neutralization Index**	**Remarks**
Neutralization Tests			
Herpes simplex	A	100	Child, non-specific encephalitis.
Herpes simplex	B	More than 50 but less than 100	Child, symptoms of encephalitis.

TABLE II
Psittacosis Complement Fixation Tests

Patient	Antigen	Titer First Specimen	Titer Second Specimen	Remarks
V.H.	"Lygranum"*	1:160 (During convalescence)	1:128 (14 days after 1st specimen)	Owned parakeet. Clinical psittacosis.
M.H.	"Lygranum"	Negative 1:5	1:20 (11 days after 1st specimen)	Owned parakeet. 3 day illness, not severe.
S.M.	"Lygranum"	1:10 (10 days after onset)	1:160 (24 days after 1st specimen)	R. R. express messenger. Exposed to birds in transit. Clinical psittacosis.
M.F.	Psittacosis	1:160	1:80 (25 days after 1st specimen)	Clinical psittacosis.
M.H.	"Lygranum" Psittacosis	1:10 1:80	1:80 (23 days after 1st specimen) 1:320 " "	Raised parakeets. Clinical psittacosis.
L.S.	"Lygranum" Psittacosis	1:40 1:160	1:160 (23 days after 1st specimen) 1:640 " "	Daughter of M.H. Psittacosis c.f. titer 1:40 3 months after second specimen.

* E. R. Squibb & Sons.

TABLE III
Selected Serologic Tests for Influenza

Patient	Influenza Antigen Type	Titer Acute Phase		Titer Convalescent Phase	
		Hemagglutination	C.F.	Hemagglutination	C.F.
A	A	1:16	1:32	**1:128**	**1:128**
	B	1:16	1:16	1:16	1:16
	A'	1:16	1:16	**1:256**	**1:64**
B	A	1:32	1:8	1:64	**1:512**
	B	1:16	1:4	1:32	<1:4
	A'	1:16	1:8	**1:256**	**1:512**
C	A	1:128	1:4	1:128	**1:64**
	B	1:32	1:4	1:32	1:4
	A'	1:64	1:4	**1:512**	**1:128**
D	A	1:256	<1:4	1:256	**1:512**
	B	1:128	1:16	1:128	1:16
	A'	1:32	1:4	**1:128**	**1:128**

Note: Convalescent sera for patients A and B drawn 21 days after acute.
Convalescent sera for patients B and C drawn 11 days after acute.
Bold Figures denote significant rise in antibody titer.

Tissue cultures[7] are once more coming into favor for growth or isolation of viruses.

Laboratory Results

Harding, *et al.*[16] performed 4,365 separate viral complement fixation tests on sera of acutely ill hospital patients, and obtained positive results in which a diagnosis of viral infection was confirmed on only 14 patients (2 for lymphogranuloma venereum, 3 for psittacosis, 2 for influenza, and 7 for mumps). On this basis, we would not have expected a high yield of significant findings in the short period we have operated a virus laboratory. The public health laboratory is also handicapped by the difficulty in obtaining paired serum specimens and information regarding the clinical status of patients.

The following tables, however, present selected reactions obtained in our laboratory which indicate the findings that can be made in a virus diagnostic laboratory. Table I presents complement fixation and neutralization data indicative of mumps, Rocky Mountain spotted fever, and herpes simplex infection. Table II gives complement fixation reactions for psittacosis on paired sera from six patients subsequently proven to have suffered from this infection. It may be noted that titers with psittacosis antigen are higher than those with "Lygranum," as would be expected in cross reactivity of the psittacosis-lymphogranuloma group. Comparisons of hemagglutination-inhibition and complement fixation tests for influenza are presented in Table III. Four-fold or greater rises in titer were found to the A-prime virus by both techniques, but the complement fixation test also indicated rises to A (PR-8) viruses in the four specimens. The fourth table indicates five influenza virus isolations, and two of psittacosis. The Delaware influenza virus was utilized by the influenza strain center as one of two representative 1953 influenza prototype viruses, which differ as much from the FM-1 strain as the latter differs from the PR-8 virus.[17]

TABLE IV

Virus Isolations

INFLUENZA

5 virus isolations from 24 throat washings

Type A-prime virus obtained from:

- Columbus (Hospital A)
- Columbus (Hospital B)
- Delaware (University Health Service)
- Wooster (Rural)
- Cincinnati (City Health Department)

PSITTACOSIS

2 virus isolations from 5 parakeets

- A. Bird from Delaware, Ohio, origin Texas.
- B. Bird from Columbus store, origin New York City.

SUMMARY

A review of viral techniques which are performed in a public health virus laboratory has been given, with illustrations of significant laboratory findings.

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A SIMPLE PYCNOMETER FOR MEASURING THE SPECIFIC GRAVITY OF BLOOD

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The specific gravity of blood, that is the ratio of the weight of the blood to the weight of the same volume of distilled water at a temperature of 4°C., may be determined directly by weighing a given volume of blood.

Schmaltz[1] used a complicated capillary pycnometric method for measuring specific gravity of blood. Hammerschlag[2] also used a pycnometer, but he measured the specific gravity of a mixture of two liquids in which a drop of blood remained suspended.

In the falling drop method of Barbour and Hamilton,[3] a 10-cu. mm. drop of the fluid to be analyzed is timed as it falls over a distance of 30 cm. through a mixture of xylene and bromobenzene in a tube of 7.5-mm. bore. Its falling time is compared with that of a 10-cu. mm. drop of a standard potassium sulfate solution of known density.

The most accepted method of measuring specific gravity is that of Phillips and coworkers,[4] where small drops of whole blood or plasma are allowed to fall into a graded series of copper sulfate solutions of known specific gravities. Upon contact with the solution each drop becomes encased in copper proteinate and remains as a discrete drop without change in specific gravity for 15 to 20 seconds.

A direct pycnometric method is as accurate and much less time consuming than any of the above methods.

We made use of the direct pycnometric analysis in our diagnostic laboratory and found it highly satisfactory.

Our pycnometers were made from 0.5-ml. pipets. These pipets are relatively inexpensive and found in ample sufficiency in most diagnostic laboratories. The pipets are heated and bent into proper shape. See Fig. 1. The insides of these pycnometers are coated with a thin film of organosilicon preparation (duPont) in order to prolong the fluidity of the blood samples. The organosilicon preparation is drawn through the bent pipet by attaching a rubber tubing to a suction apparatus. Care is taken not to draw the organosilicon compound into the rubber tubing. This coating, which cannot be washed off by ordinary washing, prevents the blood from coagulating for 12 to 15 minutes.

* Present address: University of Cincinnati School of Medicine, Cincinnati, Ohio.

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A piece of wire is bent and fastened to each pipet, as shown in Fig. 1, to facilitate weighing. The bent pipets are recalibrated with distilled water, and if necessary the new graduation is marked. This mark can be etched on with hydrofluoric acid if desired.

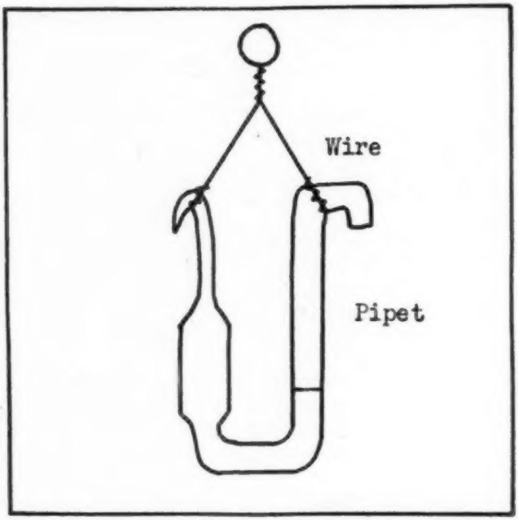

Fig. 1—Pipet, 0.5-ml. capacity, bent to form a pycnometer.

The pycnometer, clean and dry, is weighed on an analytical balance. A freshly drawn sample of blood, with no anticoagulant added, is carefully drawn just to the proper point on the pipet. The weight of the sample would be slightly altered by that amount of blood which clings to the inside of the pipet if the blood is aspirated above the mark. The outside of the pipet tip is carefully wiped clean with a lint-free gauze. The difference in weights of the empty pycnometer and the sample plus pycnometer is the weight of 0.5 ml. of blood. This figure multiplied by 2 gives the correct specific gravity for that sample of blood. Using a smaller pipet for a pycnometer would be sacrificing accuracy in order to keep the sample size small.

The pycnometer should be immersed in water as soon as weighing is finished to prevent blood from adhering unnecessarily to the inside of the bent pipet.

Pycnometers which are difficult to clean with water and a soapless-detergent can be left in a chromic acid-cleaning solution

overnight. This treatment will provide clean pipets and will not alter the organosilicon coating. The wire holders used to suspend the pycnometers on the balance should be removed during the cleaning process.

We found our pycnometric method compares well with the copper sulfate-suspension method of Phillips and coworkers.[4] See Table I for a comparison of the two methods.

TABLE I

Comparison of Phillips' Copper Sulfate-Suspension Method and the Direct Pycnometric Method of Determining Specific Gravity of Blood

Sample No.	Copper Sulfate Suspension (Sp. Gr.)	Pycnometer Method (Sp. Gr.)
1	1.060	1.0596
2	1.060	1.0588
3	1.056	1.0555
4	1.054	1.0548
5	1.052	1.0517
6	1.050	1.0492
7	1.050	1.0490
8	1.048	1.0482
9	1.040	1.0398
10	1.036	1.0352

The normal specific gravity of blood is given as 1.048 to 1.066, with averages of 1.052 to 1.063.[5,6] It is slightly higher in men (1.057) than in women (1.053). There is a normal diurnal variation of about 0.0030, the specific gravity being generally lower in the afternoon and after meals and higher after exercise and during the night.[7]

The specific gravity of the blood depends upon a number of factors, especially the hemoglobin content of the red cells and the protein content of the plasma. Since these are usually measured independently, few have been interested in studying the specific gravity of blood in various disease conditions. There is a linear relationship between the plasma specific gravity and the total protein content.[8]

We found that most cases of severe degenerative diseases, cancer, and related conditions have a specific gravity of 1.070 or above. A total of 1,000 pathological blood samples were analyzed for their specific gravity. See Fig. 2. This rise in the specific gravity may be due to an increase in one or more of the protein constituents of the plasma. Also, we found an abnormally low specific gravity in cases of deficiency anemias, excess hemorrhage, and microcytic anemias which is due to a decrease in the hemoglobin content of the erythrocytes or decrease in the number of erythrocytes.

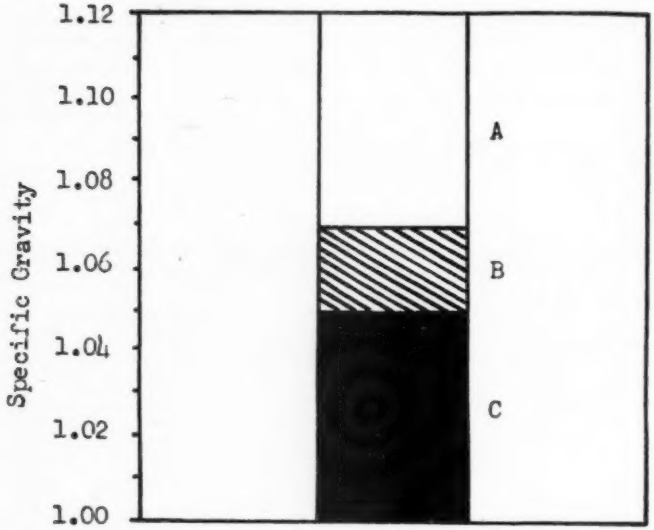

Fig. 2—Distribution of specific gravities of blood in random samples. A) Cancer and degenerative diseases, 301 cases. B) Normals and various acute conditions, 456 cases. C) Deficiency anemias, severe hemorrhage, microcytic anemias, 243 cases.

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REPRINTS FROM STATE JOURNALS

With this issue of the *American Journal of Medical Technology*, we begin a new section which will be made up of reprints of some of the best articles appearing from time to time in the state journals being published throughout the country.

The articles selected will, of course, all pertain to things of interest and value to all medical technologists, things that will help them in the performance of their duties, things that will help to simplify their work and conserve time and energy; some of the articles may give us references to some of the new books coming out in our field of work; others will call attention to new equipment and new methods that have been successfully and satisfactorily tried out in other laboratories and any original ideas or inventions which individual technologists may be working on or are using in their own laboratories; also things that may give us new ideas for better co-operation and relationships among the technologists themselves and with other people working in related fields of work. Sometimes we may add a bit of interesting poetry written by some of our own members or maybe a crossword puzzle; in fact, any little bits of interesting information that we may find in these journals. In general—this will be a section for sharing ideas so that we may all profit by the experiences and ideas of our fellow-workers.

And—we'd like a name for this new section, and would like technologists throughout the country to join in helping us to select one, so anyone having an idea or suggestion for a name, please send it in to the Editorial Office of the Journal, Hermann Professional Bldg., Houston 25, Texas.

Esther Lemont

From The Techni-caller Arkansas Society Winter 1953 Vol. 2 No. 4

CARE OF THE MICROSCOPE

ALLEN S. WEINTRAUB*

In response to numerous requests, I will attempt to outline a few pertinent features in regard to care and maintenance of microscopes. Successful microscopy requires skill and proper care of the instrument. The microscope is a precision instrument—with reasonable care it will last over a life time—but a single bit of carelessness may ruin it. The lenses being the most important part of a microscope they must be kept scrupulously clean. To remove surface dirt or dust from the optical elements, I have found that the use of a soft and dry camel's hair brush performs a wonderful job in loosening these particles, followed by the use of a good grade of lens tissue. Lens tissue that is smooth, dense and thin should be used—in view of the fact that it is highly absorbent, and because of the smooth dense structure, it cannot harbor dust particles which may scratch delicate lenses and other highly polished surfaces. Lens tissue that is soft and porous should not be used—because optical glass is softer than window glass and is easily scratched.

Dust on ocular lenses can easily be seen as specks when you rotate the ocular while looking through the microscope. Specks of dirt which may adhere to the optical surface after the use of the camels hair brush and lens tissue can be removed with a soft linen cloth which is dampened with distilled water—in the case of oil immersion objectives, xylene can be used—bearing this in mind—use no more liquid than the least possible

* Allen Weintraub has proven himself many times over an untiring friend of The Arkansas Society of Medical Technologists. He has been a vital force in the promotion of several major projects of the group. The Doctor-Technologist Team relationship and the public relations of the society have definitely benefitted by his words and deeds. If we had honorary members in tne society, Allen Weintraub would be high up on the Arkansas list.

and wipe dry with fresh lens paper. Do not soak any of the lenses in xylene or any other solvent, because it will injure the mounting of the lenses if it gets beyond the seal of the front lens into the objective. Most microscopists have a tendency to blow their breath on lenses to remove dust particles—this should be avoided because it will cover them with minute drops of saliva which are removed from the lenses with difficulty.

In regard to the rack and pinion assembly these should be wiped occasionally with a cloth moistened with a light grade of acid-free machine oil. Most oils contain acids which tend to wear on the slideways of the microscope. Too much oil will collect dust and gum as it dries.

In conclusion, the rest of the microscope should be kept free of dust—especially when it is not being used. Place the microscope under a good cover or replace it into its case.

From The West Virginia Microscoop Jan. 1954

HELPFUL HINTS

Time Saver:

Plastic 500 cc. bottles with a Deeminac filter cap are excellent time savers for quick rinsing of absorption cells and flame beakers right beside the Spectrophotometer.

Reference: St. Luke's Hospital Laboratory, Bluefield, W. Va.

Histology:

To prevent paraffin dripping down over base of bunsen burner while heating paraffin tissue block holders, pinch a hole in the middle of a piece of heavy paper and place over the stem of the bunsen burner. The paper catches the melted paraffin instead of the air vent or base of the burner.

Reference: Patti Bell, Myers Clinic Hospital Laboratory, Philippi, W. Va.

Suggestions for Blood Donors:

If you like to have the donor pump his hand to accelerate blood flow, a soft, medium-sized sponge will prevent his hand getting tired quickly or the fingernails digging into the palm.

Reference: St. Luke's Hospital Laboratory, Bluefield, W. Va.

From Alabama Society of Medical Technologists Newsletter

Feb. 20, 1954

IT'S GREEK TO ME

The difference between a technician and a technologist is explained in the "ologist." "-ology" means the discourse or ability to think and reason; the study of. Therefore, a technologist is one who has made a study of an art or skill whereas a technician is merely one who performs such art of skill. We are Medical TechnOLOGISTS; let's let people know it.

EDITORIAL

LOOKING BACKWARD HELPS

Youth is impatient. Age reflects.

Youth is "rarin' to go." Age pauses, meditates, examines and chooses a focus.

Youth asks, "Why can't we go farther, faster?"

Age says: "See how far we have come in a short time. 'Rome was not built in a day'."

And the miracle lies in the fact that both points of view are necessary for balance, growth, equilibrium and progress.

Some of us in the "age" group feel that it is a privilege to be able to look back over a period of time and KNOW what has actually taken place, and feel it would not be amiss to share a bit of this knowledge with Youth. Far be it from us to say: "Hold your horses." Rather let us say: "Don't be discouraged when the changes you want come slowly." We

ARE "getting there." We HAVE advanced. Yes, we have advanced steadily, slowly and surely. See how our status compares now to our "childhood days," professionally, scientifically, organizationally, and economically.

Only remember, when we help you to "look back" that to us, thirty to thirty-five years is really a SHORT time. The profession of Medical Technology was a babe-in-arms in 1920. Florence Nightingale established the career of nursing with her assignment in the Crimean War in 1854!

FLASH BACKS!

Prior to 1928. Professional status of a clinical laboratory worker—zero! No standardization of educational requirements. No regulated courses or approved schools for learning laboratory technique. No recognition professionally at all.

1930. Average base pay of an M.T. around $100.00 a month (often less). Many hours of "call." Over-time rarely compensated for by either "time off" or extra pay. "Call" and extra week-end hours all taken for granted. 44 to 48-hour week customary.

Prior to 1933. No A.S.M.T. in existence. No state societies. A few scattered local groups, ineffective in any matters requiring unified action. No American Journal of Medical Technology. No state journals or Newsletters. One end of the country didn't know what the other was doing. No conventions, no exhibits. Only a very occasional scientific paper being written by a Medical Technologist.

1935. Quoted from the Registry report of July 1935. "The unprecedented number of **464** applicants took the last semi-annual examination."

1937. Excerpts from A.S.M.T. Convention reports:
Membership Committee 1936-37. A.S.M.T. had 486 members.
Convention Registration. 84 A.S.M.T. members present at 1937 convention.
(Affiliation of states was in progress and this represented 26 states.)
Treasurer's report. Receipts and Disbursements approximately $1,700.00. Financial report of the Journal. Receipts and Disbursements about $3,000.00.

1945. M.T.s tried to get recognition in Washington to get commissions in the army and navy. Failed in passage of bill H.R. 3147.

Prior to 1949. No A.S.M.T. Public Relations or Recruitment Committees. No voting representation on Board of Registry or Board of Approved Schools.

LOOK AT US NOW. 1954

Professional recognition by A.M.A. and other medical organizations. Average base pay more than doubled on a 40 hour week with little or no call work. Compensation for overtime hours. Working conditions vastly improved. ASMT membership 5,500. 50 affiliated societies. 35 state Newsletters or Journals. Convention registration nearing 1,000 with scientific papers and exhibits largely contributed by the medical technologists themselves. Nearly 3,000 registry examinations given in 1953. Journal expenditures and receipts around $12,000.00. ASMT budget based on annual income $40,000.00. Commissions as laboratory officers in the armed forces due to passage of bill H.R. 4384 (1950). Voting representation on Board of Registry and Board of Approved Schools. Thousands being spent on active recruitment and public relations programs.

How could we have accomplished all this without the enthusiasm, energy and impatience of Youth—without the doggedness, judgment and experience of Age. There's a lot to be done. We've a long way to go. Progress doesn't just "happen." Hundreds have worked hard, are working hard and will continue to work hard. Hundreds more will passively reap the benefits of this work. But don't get impatient. Don't get discouraged. The turtle beat the hare. Just keep a-plod-plod-ploddin' along.—F.H.C.

AMONG THE NEW BOOKS

A PRACTICAL MANUAL OF MEDICAL AND BIOLOGICAL STAINING TECHNIQUES: By Edward Gurr, F.R.I.C., F.R.M.S., F.L.S., Interscience Publishers, Inc., New York, London, 1953, 320 pages. Appendix. $4.00.

The author has included not only the standard staining techniques but has incorporated many of the unusual as well, thus making possible a quick reference to information on the application of microscopic stains. The first section (on Fixation and Fixatives) gives a brief summary of when to use WHAT, and WHY to use it. The appendix includes a section giving the formulae for the fixatives. Although we might have organized the subject in a single section, there should really be no complaint. The material is all there, in a single volume.

Section Two gives the stain, purpose, solutions required and technique. Again, the formulae for the stains are given in the appendix. This section covers normal and pathological animal histology. Section Three is on Botanical Methods, with a few pages on miscellaneous tests as well as the bacterial stains. The Fourth section is on Cytological Methods, and is the least valuable, while that on Fluorescence Microscopy, although brief, is good reference material. Section Six, on Staining Bacteria, will make the book worth having in the clinical laboratory and in the training school.

DISORDERS OF THE BLOOD. 7th Edition. By Sir L. E. H. Whitby and C. J. C. Britton. 856 pp., 20 color plates, 106 figures. New York: Grune and Stratton, Inc., 1953. $9.50.

This well-known and important English textbook on hematology has earned a wider and well-deserved acceptance in this country since the first edition appeared in 1935. The seventh edition (1953) of 856 pages includes numerous valuable additions of subject matter and technics which brings it thoroughly up to date.

The chapter dealing with blood groups and immunohematology is so comprehensive and well written that it could serve as a textbook in itself.

The addition of "The Biochemistry and Cytochemistry of Hemopoeisis" should stimulate further research in this interesting and important phase of hematology and is well supplemented in the technical section with methodology.

The recent advances in the blood coagulation factors have been brought together in concise fashion to give a comprehensive view of the various phenomena involved.

References to authors are liberally included in the text and supplemented by complete bibliographies at the end of each chapter, which should prove an invaluable aid to research workers and teachers.

The color plates have increased in number but could be improved with higher magnification to give better cytological detail, however those in black and white are excellent. The paper and type are superior.

The technical section, which is wisely compiled separately from the text, will appeal strongly to us, as medical technologists, and to those of us who are teaching hematology to prospective medical technologists. Such a simple reason for using an extended mouthpiece for collecting counts is that it "allows the pipette to be held in a horizontal position so that the amount entering it can be observed accurately, and that the operator has both his hands free for manipulation." The health of the medical technologist is safeguarded by providing a greater distance from the patient. This can be accomplished by uniting two standard mouthpieces.

ELSA S. KUMKE, M.T. (ASCP)

CLINICAL DIAGNOSIS BY LABORATORY METHODS—A Working Manual of Clinical Pathology. By James Campbell Todd, Ph.B., M.D., Late Professor of Clinical Pathology, University of Colorado School of Medicine; Arthur Hawley Sanford, A.M., M.D., Emeritus Professor of Clinical Pathology, The Mayo Foundation, University of Minnesota; Benjamin B. Wells, M.D., Ph.D., Professor of Medicine, Department of Medicine, University of Arkansas School of Medicine. New, 12th Edition. 998 pages with 946 illustrations, 197 in color, on 43 figures. Philadelphia and London: W. B. Saunders Company, 1953. Price $8.50.

Another edition of the volume that is a traditional part of every laboratory is still the practical, essentially elementary, basic book of methods for use of the medical technologist, the student, and the physician. The chapters on hematology and bacteriology have been revised to bring them up to date. The section on serologic tests for syphilis has been modified by bringing the various methods to conform with those published by the U. S. Public Health Service. There has been little alteration in the appendix, listings of laboratory findings in disease, and in the data on weights, measures, and solutions. To keep up to date, it is essential that each laboratory have its TODD & SANFORD, the latest edition.

TISSUE CULTURE—The Growth and Differentiation of Normal Tissues in Artificial Media. By E. N. Willmer, M. A., Sc.D., Fellow of Clare College Cambridge. 2nd Edition. 175 pages, 2 plates, 8 text diagrams. London, Methuen & Co., Ltd., New York, John Wiley & Sons, Inc. 1954. $2.25.

This pocket-sized volume may be considered a handbook on tissue culture. In two sections, the first is on UNORGANIZED GROWTH, gives the various culture methods, characteristics of growth, cell colonies and media, and metabolism of growing cells, and the second is on ORGANIZED GROWTH, and gives the differentiation and cell types. The list of journal references includes 410 titles, and there is a supplementary list of reference books with eleven titles. There is also a six page glossary.

TOXICITY OF INDUSTRIAL ORGANIC SOLVENTS—Preventive Measures for and the Treatment of Industrial Poisoning. 2nd Ed. By Dr. Ethel Browning, of the Medical Research Council, England. Chemical Publishing Company, New York, N. Y. 1953. 411 pages, 53 tables, references, and detailed index. $8.00.

The medical laboratory in an industrial plant especially, will find this book of great value. There are chapters on the Hydrocarbons, alcohols, ethers, esters, ketones, glycols, and other miscellaneous compounds. The reports give the properties, uses, toxicity, toxic effects on animals and on man. The effects on the blood, metabolism, skin, etc. are given in detail. The laboratory tests involved are described in detail, together with the possible results. Although it would not be used routinely, the information here would be extremely valuable in those instances where it applies specifically.

CONVENTION ANNOUNCEMENTS

MIAMI BEACH AND GREATER MIAMI IN JUNE

The 22nd Annual Convention of ASMT at Miami Beach, Florida, will begin June 12 at 2:00 P.M. with registration in the lobby of the Delano Hotel. Registration will continue until 5:00 P.M. and will be resumed Sunday at 8:00 A.M. The Advisory Council meeting will begin at 9:30 A.M., by request. A church directory will be available at the registration desk.

At 1:30 P.M. (Sunday) you will have a choice of one of three tours—two by land and one by sea. There is a grand or combined tour which includes the beaches, Hialeah Race Course, a Seminole Indian village, the University of Miami campus, Coral Gables, the City Beautiful, and the Orange Bowl among other places. All of this takes approximately four hours. The second trip by land is somewhat shorter, taking about 2¾ hours, and covers the beaches, Hialeah and the International Airport. The boat cruise will take you through the inland waterways and give you a

glimpse of the lovely homes on the man-made islands surrounding Miami Beach, which is itself an island. Hialeah, besides being what many consider the most beautiful race track in the world, is the home of a flock of flamingoes, that striking pink-feathered bird of the tropics. They live on islands in the lake made for them in the centerfield of the track. The University of Miami's new campus is a triumph of modern architecture and has been built, entirely, since World War II. International Airport is one of the largest and busiest in the United States.

On Sunday night at 8:00 the Florida Division of ASMT invites all to attend the reception to be held on the Terrace at the Delano Hotel.

Monday, June 14, at noon we hope you will enjoy the Cabaña Luncheon and Watershow at the Shelborne Hotel. Here you may see pool and ocean at the same time. And do bring your sunglasses—that sun can be mighty bright during the day!

For those of you not attending the House of Delegates sessions, Tuesday afternoon and Wednesday morning will be free. Arrangements may be made for any of the aforementioned tours or for any of the many other interesting trips available—Villa Vizcaya, Parrot Jungle, the Rare Bird Farm, Monkey Jungle, etc. Or maybe you'd like to go deep-sea fishing. That, too, can be arranged. And there is always shopping on famed Lincoln Road. But be sure you finish these extra-curricular activities before Wednesday afternoon because we think you won't want to miss the Hawaiian Luau (feast) at Crandon Park. Wear your bathing suits under your shorts or slacks if you want a swim, but take along a jacket for it can get cool after the sun goes down. There will be a full moon—and a surprise.

The banquet, which is to be INFORMAL, will be at 8:00 P.M. Thursday in the ballroom of the beautiful (and brand new—it opened in December) di Lido Hotel. As you will note elsewhere, Dr. H. W. Brown will be our speaker. This ends what we are confident will, with your participation, be one of the most successful conventions ASMT has ever held.

Mrs. Maxine Ace
Mrs. Anna Louise Rundell
Co-chairmen of Publicity

GENERAL INFORMATION

Florida in June is sunwarmed to about 80° with a pleasant ocean breeze to stir the palm fronds. In order to enjoy this to the fullest, bring informal clothes, cottons and comfortable shoes for the women and slacks and sport shirts for the men, sun-glasses for all. Since the hotels all have pools as well as the ocean, you'll want to bring bathing suits. And don't forget the shorts, slacks or playsuits for the Crandon Park trip. But PLEASE, do be cautious in acquiring that sun-tan! It is not at all unusual for visitors to be hospitalized with sunburn. And before we forget—since the hotels and Auditorium are air-conditioned and the evenings sometimes cool, you may wish to bring a sweater or a light jacket.

Since Miami Beach is an island it has no stations, whether you arrive by plane or train, your destination will be Miami. There is limousine service from the Airport to the Beach. This is more reasonable than taxi. From either of the two train stations, taxis are available.

Or are you planning to come by car? Route 41 comes down the West Coast through Land O'Lakes, Sulphur Springs, Tarpon Springs (sponge fisheries), St. Petersburg, Tampa, Sarasota (winter quarters of Ringling Brothers Circus, also home of the Ringling Art Museum) and Fort Myers (where you can see Edison's workshop); then across the Tamiami Trail through the heart of the Everglades and so to Miami. The most scenic route, perhaps, is 441-27 and 301 through the central part of the state, where you will find Ocala and Silver Springs, Winter Haven and the Bok Tower, the orange groves and Cypress Gardens. Or perhaps you

prefer Route 1 through Jacksonville; St. Augustine, the oldest city in the United States; Marineland between St. Augustine and Daytona Beach; and the famous beach at Daytona Beach itself. If you have your car you will want to go on South from Miami to Key West over the famous Overseas Highway. It is here you will reach the Southern-most point in the United States, only a short distance from Cuba.

Would you like to go to Cuba, Nassau, Jamaica, Hati, or Puerto Rico? Just read all the forms and check in the appropriate places and information will be sent to you. We want you to enjoy your Convention and then go on to enjoy a tropical vacation. But beware—you'll get sand in your shoes and want to come back.

SCIENTIFIC - TECHNICAL EXHIBITS

Please reserve booth space for your scientific or technical exhibit as early as possible. Prepare your exhibit for the 1954 ASMT Convention and write to Miss Emelia M. Lanzetta, 3214 Liddy Avenue, West Palm Beach, Florida, for a reservation.

The backwall of the booth will be blue-gray flameproofed draped material with side dividers covered with the same material as the backwall. A table is furnished. Covering for the table is to be furnished by the exhibitor.

Instructions for shipping will be sent after the requests are received.

Please check the following and return to Miss Lanzetta at the above address.

1. Size of booth.	All booths are 10 ft. x 10 ft.
2. Counter table.	Needed: Yes No
3. Chair.	Needed: Yes No
4. Electric outlet.	Indicate if needed for display.

One will be furnished if indicated. Additional outlets $7.50.

5. Headboard sign.	9 in. x 44 in. One or two lines to read

PLEASE PRINT

Applicant responsible for exhibit: ______________________

(Signature)

N.B. Please print:

Name of Applicant
Address of Applicant
Type of Exhibit

SPECIAL FOR THE SISTERS

A special trip has been arranged on Sunday to give you a chance to see Mercy Hospital, which is located on part of the old Deering Estate and faces Biscayne Bay, and Villa Viscaya, former home of James Deering. Villa Vizcaya, now an art museum, is modeled from a 16th Century fortress-like structure and is surrounded by Italian gardens. It houses a wonderful collection of paintings and art objects from far-away places. Mr. Deering traveled for 25 years through Europe collecting rare textiles, period furniture, sculpture and ceramics. The Italian art is unexcelled in America.

On Wednesday St. Francis Hospital has planned a buffet dinner followed by a movie. This hospital has a fine view of Indian Creek as well as the ocean, and is situated on Allison Island at 63rd Street, Miami Beach.

HOUSING FORM FOR SISTERS

Date of Arrival:________________________________

Time: A.M.__________ P.M.__________ After 6:00 P.M.__________

Date of Departure:________________________________

I would like: Convent Accommodations____________________

Hotel Accommodations____________________

Name:________________________________

Address:________________________________

Please return as soon as possible to:

Sister Evelyn Marie
St. Francis Hospital
Miami Beach, Florida

NOTICE

Guests (family or friends) will be welcome at any of our entertainment features. However, at any event or on any tour where space is limited, members and visiting technologists will be given preference, and guests will be accommodated in the order in which their reservations are received. Our Housing Committee will be glad to help in finding accommodations for your guests. Please be sure to indicate clearly on all reservation forms whether they are for members, non-member technologists, family or friends. We sincerely hope we will be able to accommodate all who wish to attend. Remember to bring your current ASMT membership card as identification. Every technologist, member or non-member, must pay the registration fee. Guests (family or friends) do not pay this fee.

SUNDAY, June 13		
1:30 P.M.	Sightseeing Tours	
	Combined bus tour	$3.00
	Short bus tour	1.73
	Boat cruise	2.15
	Special trip for Sisters, including entrance fees for Villa Vizcaya	3.30
MONDAY, June 14		
Noon	Cabana Luncheon	3.50
WEDNESDAY, June 16		
4:30 P.M.	Hawaiian luau, including transportation	4.50
4:30 P.M.	Special for Sisters: Buffet supper and movie at St. Francis Hospital, transportation only	0.50
THURSDAY, June 17		
8:00 P.M.	Banquet, di Lido Hotel	7.50
REGISTRATION FEE		3.00

ADVANCE REGISTRATION WILL CLOSE JUNE 4, 1954.

After that date you may register on your arrival at the Convention.